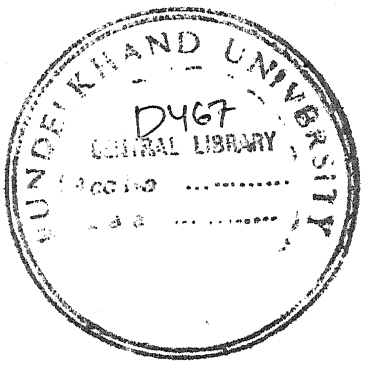
A Study of the Relationship Between Suxamethonium Apnoea and Serum Cholinesterase in the Local Population

A Thesis for

Doctor of Medicine

(Anaesthesiology)



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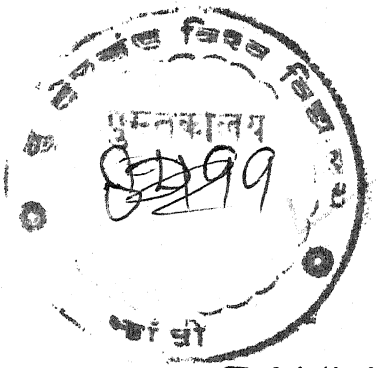

RAVI KANT SHUKLA

C E R T I F I C A T E

This is to certify that the work "A STUDY OF THE RELATIONSHIP BETWEEN SUXAMETHONIUM APNOEA AND SERUM CHOLINESTERASE IN THE LOCAL POPULATION", which is being submitted as a THESIS for M.D. (Anaesthesiology), was carried out by Dr. Ravi Kant Shukla under my personal supervision and guidance. The techniques and methods described were undertaken by the candidate himself and the observations recorded have been periodically checked by me.

He has put in the necessary stay in this department as required by the regulations of Bundelkhand University.

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ACKNOWLEDGEMENTS

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Today, when I pick up my pen to express my heartfelt thanks to all those who helped me realise what I consider so dear, I have no dearth of feelings but only an understanding of the futility of my expression. For, I am sure, I can never manage to bring forth my sincere gratitude towards all who have meant so much in the formation of this project. Yet, I shall try.

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Although friends perhaps do not need these words, but I shall be failing my duty by not mentioning them. Dr. R. K. Sharma, Dr. M. C. Misra, Dr. Tirlochan Singh, Dr. P.K.S. Chauhan and Dr. Anil Sachdeva, all helped me at various stages of my work. Their help is unaccountable.

I dedicate this work to the love, understanding and patience of my parents and other family members, which has sustained me throughout.

The manuscript has been typed in an exemplary manner by Sri P. C. Sachan. I am sincerely thankful to him for his untiring efforts in printing this work neatly in black and white.

Ravi Kant Shukla

(Ravi Kant Shukla)

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INTRODUCTION

I N T R O D U C T I O N

Scientists have been fascinated by muscle relaxant drugs ever since the discovery of use - by the Indian of the Amazon Basin - of poisoned arrows. These animals fell down alive, but were unable to run even if the trauma was trivial. This, perhaps, was the first unsubstantiated clinical impression related to relaxant - drugs.

Today, muscular relaxation is imperative for most surgical procedures. Previously, adequate relaxation could only be produced by deep planes of General Anaesthesia or various procedures of regional block.

In modern clinical practice a number of relaxant drugs are in use - all having their limitations, advantages and disadvantages. Introduction of these drugs has been hailed as one of the greatest advances of this era.

Thanks to these drugs, apart from endotracheal intubation and suction, today Anaesthetists can have full control of patient in situations that previously used to be disastrous. The relaxants have also significantly contributed to the technique of "balanced anaesthesia" (Lundy, 1942) which has brought pleasure and safety to the administration of anaesthesia. No more are the deep planes of anaesthesia necessary for the much needed relaxation. Today our surgical colleagues take all this for granted.

Further, by eliminating the work of breathing,

relaxants actually produce therapeutic relief in patients in shock and low general condition. In addition, Intermittent Positive Pressure Respiration (I.P.P.R.) if correctly given, can produce better ventilation than spontaneous breathing.

The introduction of suxamethonium in clinical practice in 1951 (Thesleff, S. ; Brucke, H. et al. ; Mayrhoffer, O. et al.) caused still further excitement due to its capabilities of producing a short - lived but total muscular paralysis in the relative absence of side - effects - thus allowing the use of larger doses of the drug.

Over the years it was proved that the short - lived action of suxamethonium was due to its rapid hydrolysis by cholinesterase. Mendel and Rudney (1943) proved that two types of cholinesterases are present in the human body :

(1) "True" cholinesterase.

(2) "Serum" cholinesterase.

This latter enzyme is responsible for the rapid hydrolysis of suxamethonium and is thus of clinical importance to the Anaesthesiologist.

Studies of the cholinesterase levels in different diseases and conditions have been carried out by various workers (Hall and Lucas, 1937; Faber, 1943; Kunkel et al.; 1947; Hutchinson et al., 1951; Vorhaus et al., 1953; Kaufman, 1954; Moore et al., 1957; Wetstone et al., 1960; Lanks et al., 1976 and Epstein, 1980).

It was found that the deficiency of serum cholinesterase may be of a quantitative or a qualitative nature. Quantitatively low values of the enzyme have been reported in association with the following diseases or conditions :-

(1) Liver diseases.

(2) Malignancies - specially of gastrointestinal tract.

(3) Malnutrition.

(4) Chronic anaemias.

(5) Organophosphorus exposure.

(6) After therapeutic radiation.

(7) After treatment with anti-cancer drugs.

(8) Mid and last trimesters of pregnancy, labour and early post-partum days.

(9) Severe dehydration and electrolyte imbalance.

(10) Acute infections.

Quantitatively raised values have also been noticed in :-

(1) Obesity.

(2) Nodular goitre.

(3) Thyrotoxicosis.

(4) Nephrosis.

(5) Anxiety states.

(6) Schizophrenia.

(7) Psoriasis.

(8) Alcoholism.

Quantitatively low values of serum cholinesterase are of significance to an Anaesthetist since in such cases the body has reduced ability to metabolize suxamethonium and consequently its duration of action may be prolonged.

Later work by Kalow and his associates over the period 1957 - 1960 brought into light a smaller group of persons who were not ill, but were qualitatively deficient in serum cholinesterase. Presence of the "Atypical" serum cholinesterase in such persons is due to inheritance of an abnormal cholinesterase gene. The percentage inhibition of cholinesterase by the local anaesthetic drug dibucaine - denoted by "Dibucaine Number (D.N.) - was shown to distinguish between persons with normal serum cholinesterase and those with the qualitatively different serum. Various types of such qualitative deficiencies were seen and recorded - a fluoride - resistant gene (Harris and Whittaker, 1961), a silent - gene (Lidell et al., 1962), a C 5 - variant (Harris et al., 1963).

Aims of this study :-

It has been shown by Evans et al. in 1952, Argent et al. in 1955 and Vickers in 1963 that the cholinesterase enzyme activity must be greatly reduced before any significant prolongation of suxamethonium effect is observed. Still later King and McQueen in 1976 stated that the lower the cholinesterase activity and the dibucaine and fluoride numbers, the more prolonged the apneic response to suxamethonium.

In the light of these facts many workers are of the opinion that quantitatively or qualitatively decreased levels of serum cholinesterase contraindicate the use of suxamethonium.

Katz (1969) found that serum cholinesterase levels were higher in London than in New York patients.

Srinivasan (1972) found a high incidence of genetic abnormality as well as low levels of serum cholinesterase in patients belonging to Bundelkhand region. Prompted by this observation the present project was undertaken with an aim to make a proper survey of this deficiency in this region and to study its relationship to suxamethonium apnoea in patients operated at M.L.B. Medical College and Hospital, Jhansi.

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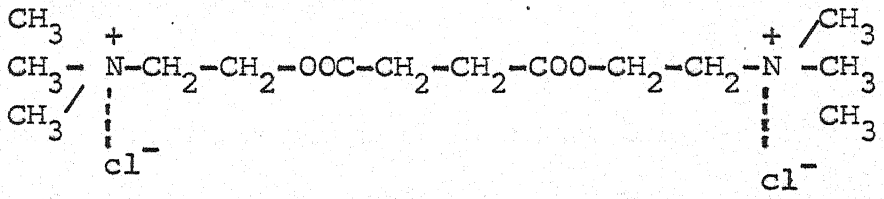
REVIEW OF LITERATURE

R E V I E W O F L I T E R A T U R E

Suxamethonium enjoys an enviable position among the drugs used in modern anaesthesia practice. It is used to produce muscle relaxation of short duration during anaesthesia.

Suxamethonium is the dicholine ester of succinic acid. It is a synthetic bis-quaternary ammonium compound with a melting point of 150°C. A white crystalline substance, it is unstable if warmed or in an alkaline solution.



Suxamethonium chloride.

History:

The pharmacological properties of suxamethonium were first described by Reid Hunt and Taveau (1906). Its rapid hydrolysis by cholinesterase in the horse serum was demonstrated by Glick (1941).

The neuromuscular blocking properties of this drug were however first described by both Bovet et al. (1949) and Phillips (1949) separately. Demonstration of the breakdown of suxamethonium by cholinesterases and the inhibition of this hydrolysis by eserine was done by Bovet-Nitti (1949).

Animal experiments conducted by Castillo and de Beer (1950) confirmed these findings.

The first clinical use of suxamethonium was by Thesleff at the Karolinska Institute in Stockholm (1951), by Brucke et al. (1951) and Mayrhofer and Hassfurther (1951) in Austria.

Normal Neuromuscular Transmission:

Function of the neuromuscular junction is much better understood than its structure. The neuromuscular junction includes both the pre- and post-synaptic areas. The latter is also known as motor end-plate.

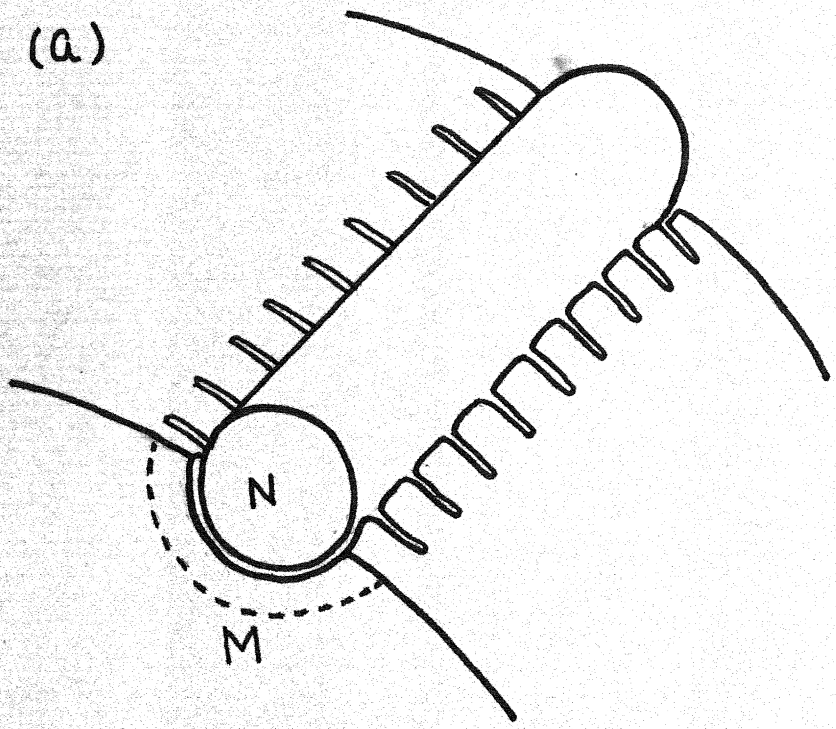
The myelinated motor nerve fibre divides into numerous non-myelinated terminal branches in the proximity of the muscle-fibres. Thereafter, these branches run parallel to the muscle fibre they supply, while lying embedded in a shallow "gutter" or depression in the muscle surface (Fig. 1).

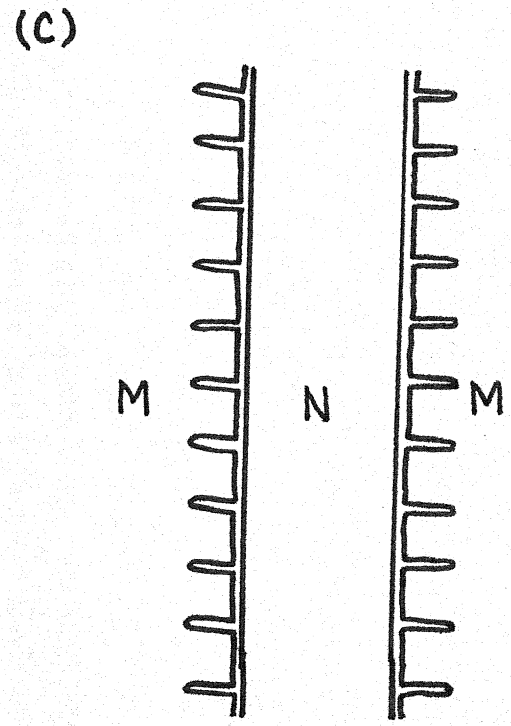
At the myoneural junction the nerve-fibre is covered by a membrane-complex known as the Schwann, axoplasmic or perineural membrane - this separates the end-plate from the extracellular fluid. As shown in Fig.2 , these layer make regular folds, towards the muscle fibre lying close to and indenting the basement membrane of the latter. This region has a high concentration of cholinesterase. The folds described are called the junctional folds or secondary clefts.

Much of the basic information about the microanatomy

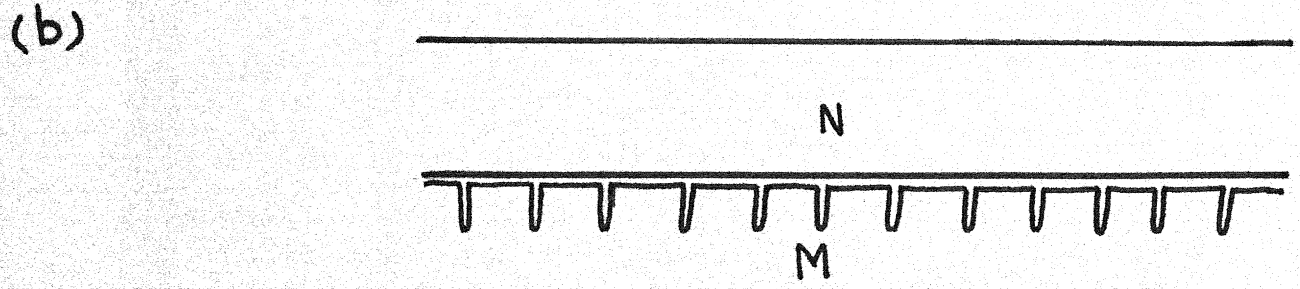
Fig. 1 - DIAGRAM OF NEUROMUSCULAR JUNCTION.

(a) Shows a small portion of the terminal axon branch N lying in a gutter formed by the surface of the muscle fibre M. The semicircular post-junctional folds are illustrated.





(c) Same in tangential section.



(b) Same in longitudinal section.

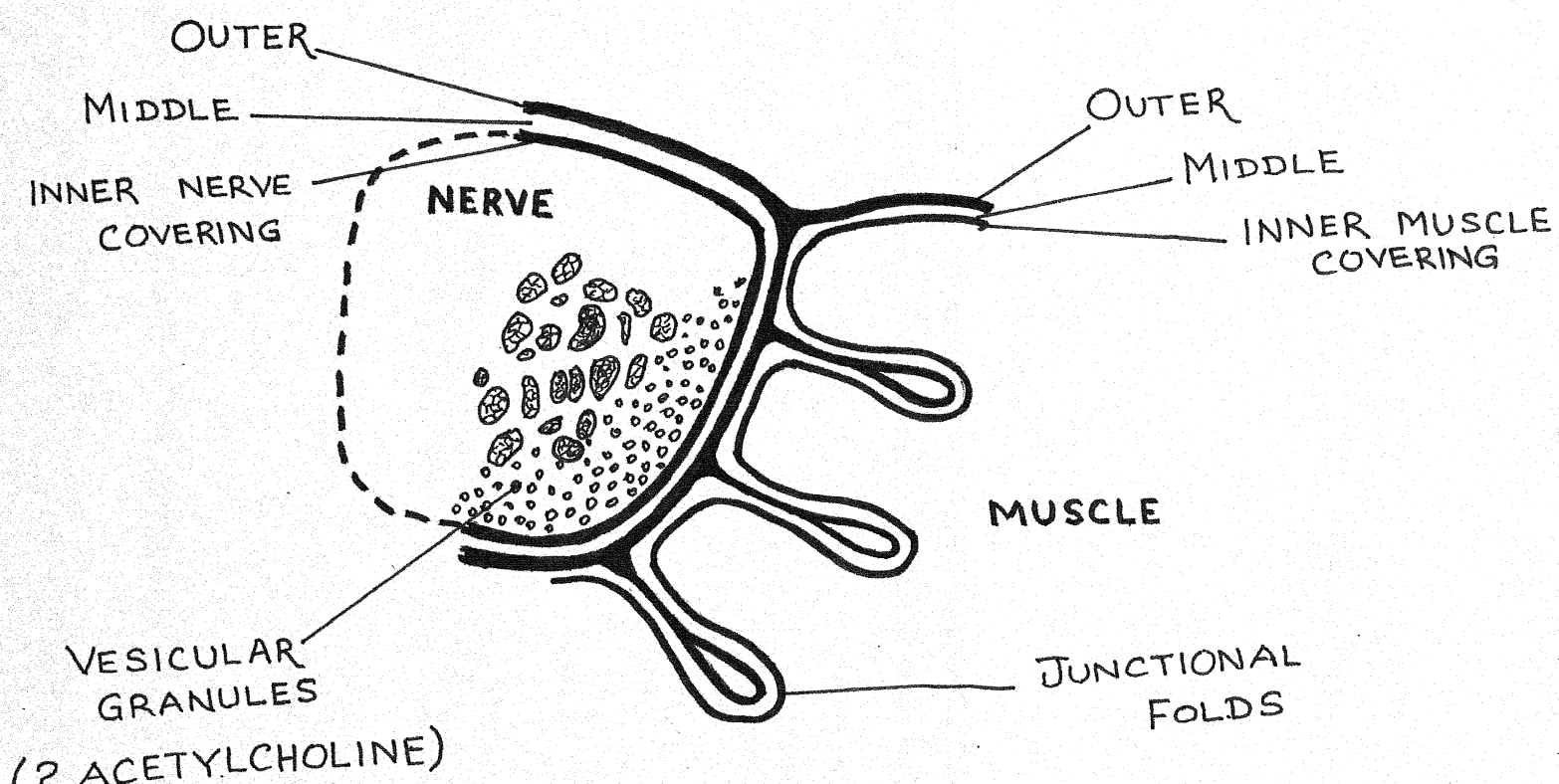


Fig. 2 - DIAGRAMMATIC REPRESENTATION OF THE NEUROMUSCULAR JUNCTION.

of the neuromuscular junction has been provided by the studies of Couteaux (1955 and 1958), Robertson (1956) and Waser (1970). It was suggested by Waser (1970) that these folds contain the sodium "pores", which allow the ionic flux, responsible for depolarization.

The narrow neck of each pore is guarded by two molecules of acetyl cholinesterase, each with two curare receptor sites. The acetyl choline receptors are scattered around the mouth of the pore. Depolarisation occurs when sufficient acetyl choline molecules combine with their receptors and cause a deformation of the surface, this pulls open the mouth of the pore, thus permitting sodium ions to pass inside the cell and result in depolarisation.

Non-depolarising drugs like curare act by combining with the active centres of the acetyl cholinesterase molecules - thus obstructing the neck of the pore.

The nature of the acetyl choline receptor is disputed. Mucopolysaccharides (Waser, 1970) , phosphate (Nastuk, 1967) or a polypeptide or protein (Gill, 1965) have been suggested.

Dale et al. (1936) propounded the chemical theory of neuromuscular transmission according the which acetyl choline bridges the gap of 1 um. between the motor nerve ending and the end-plate. This choline ester is synthesized and stored in the motor nerve ending in the form of vesicles containing quanta or packets of the substance. The vesicles have been

demonstrated in the region of nerve-ending by electron microscopy. On the arrival of an impulse the acetyl choline is released. (Feldman, S., in Wylie and Churchill-Davidson, A practice of Anaesthesia).

When sufficient molecules of the transmitter substance reach the end-plate, a threshold depolarisation results. This causes a wave of depolarisation or propagated action potential along the entire length of the muscle fibre with resulting mechanical contraction in its wake. The acetyl choline molecules excite the end-plate in the fraction of a millisecond before they are destroyed by cholinesterase within $1/500^{th}$ of a second (Feldman, S., in Wylie and Churchill-Davidson, A practice of Anaesthesia).

Neuromuscular block caused by suxamethonium:

A depolarising block is produced by suxamethonium. This is similar to the block caused by acetyl choline except for the greater extent and duration of the depolarisation. It was originally believed that this type of block was due to a lowering of the trans-membrane potential to a level which prevented initiation of the propagated action potential. Evidence towards this concept was advanced by Jenerick and Gerard (1953) and del Castillo and Katz (1956).

Katz and Thesleff (1957) discovered that loss of sensitivity of the membrane far exceeded the time for which it remained depolarised. This phase was termed by them as

the 'desensitisation phase' of depolarising neuromuscular block. Feldman and Tyrrell (1970) postulated that the determining factor as to whether a neuromuscular blocking drug acted as a depolarising or non-depolarising agent was whether or not it became bound to the receptor. A depolarising block is preceded by muscle fasciculations.

It has been demonstrated that prolonged administration of a depolarising drug (Jenden et al., 1951; Brennan, 1956) or its use in excessive doses produces a change in the nature of the neuromuscular block from a depolarising type to one with non-depolarising characteristics. Zaimis (1953) noted this in chicks and coined the term "dual block" for this phenomenon.

Zaimis et al. (1952) and Zaimis (1953) demonstrated the different responses to decamethonium in different species - a depolarising block was produced in some while others developed a block resembling a non-depolarising one. Churchill-Davidson and Richardson (1952) found electromyographically that an identical change in decamethonium activity occured in patients of myasthenia gravis. Churchill-Davidson and Wise (1964) discovered that a similar block occured in premature infants when depolarising drugs were administered to them. Churchill-Davidson et al. (1960) saw that this type of block could be produced on giving excessive doses of suxamethonium . It was found by Foldes et al. (1956), Argent et al.

(1955) and Brennan (1956) that this type of block could be reversed by neostigmine or edrophonium.

It is considered better to refer the block as occurring in two stages - Phase I, which is potentiated by anti-cholinesterase drugs and by tetanic stimulation and Phase II, which shows characteristics of a non-depolarising block. In the latter phase a significant, though at times short-lived, reversal occurs on administration of an anti-cholinesterase. Tetanic stimulation produces some degree of post-tetanic facilitation and tachyphylaxis is seen to further doses of the depolarising drug.

Feldman and Tyrrell (1970) were of the opinion that the molecules of the depolarising drug became bound to the receptors in an increasing proportion, thus producing a significant degree of receptor occupancy; this resulted in the development of a non-depolarising component to the neuromuscular block. Galindo (1971) proposed a pre-synaptic site of action of the depolarising drug to be responsible. Despite these explainations it must be stated here that the cause of the Phase II block is still not known.

Duration of action of suxamethonium:

A single injection of suxamethonium (50-75 mg.) produces, on an average, respiratory paralysis for 2-4 minutes (Evans et al., 1952). These workers also demonstrated the close correlation of the duration of action of suxamethonium with the serum cholinesterase levels in the individual. If a

period of apnoea exceeds 10 minutes in the absence of any other factor which might cause the prolongation of action, it should be considered an abnormal response. (Feldman, S., in Wylie and Churchill-Davidson, A practice of Anaesthesia).

Evans et al. (1953) established that by prior raising of the cholinesterase level artificially, it was possible to shorten the apnoea produced by subsequent doses of suxamethonium.

The enzyme preparation cholase, which was a concentrated human globulin containing cholinesterase, was effective only when it was given before or simultaneously with suxamethonium (Goedde et al., 1967). Clinical experiments to terminate a prolonged apnoea by intravenous infusion of concentrated cholinesterase were not successful.

The highly purified concentrated preparation of cholinesterase (Doenicke et al., 1968; Goedde and Atland, 1971) was successful in terminating a prolonged apnoea within 8 minutes after its intravenous injection. Doenicke et al., (1968) showed that with a relatively small dose of 30 mg. of this cholinesterase preparation, a shortening of the duration of apnoea following 50-200 mg. suxamethonium was obtained. After 50 mg. suxamethonium the apnoea could be entirely neutralised.

Suxamethonium dissociates rapidly from the end-plate receptors and diffuses back into the plasma where it is hydrolysed by the commercial cholinesterase infused. The upset equilibrium causes more dissociation of suxamethonium and

repetition of this process ultimately results in termination of the apnoea (Stovner and Stadskleiv, 1976).

Doenicke et al. (1968) have shown serum cholinesterase inhibition due to suxamethonium itself. Their in vivo experiments demonstrated that this enzyme inhibition was directly related with the duration of apnoea. The emergence of the patient from suxamethonium apnoea occurred as soon as no further enzyme inhibition was measurable.

Detoxication and Excretion of suxamethonium:

Suxamethonium is well known for its short duration of action. The short duration is due to its rapid hydrolysis by the enzyme serum cholinesterase. This process occurs in two stages:-

1st Stage:

$$\underset{\text{(Suxamethonium)}}{\text{Succinyldicholine}} \xrightarrow[\text{Serum cholinesterase}]{\text{(Rapid)}} \underset{\text{choline}}{\text{Succinylmono-}} + \text{choline}$$

The hydrolysis commences within seconds of the entry of suxamethonium into plasma. It is so rapid and effective that less than 5 % of the injected dose reaches the muscles in the periphery and less than 2 % appears in the urine (Foldes and Norton, 1954). As has already been mentioned, as soon as the suxamethonium-induced depression of serum cholinesterase ends, spontaneous respiration returns (Doenicke et al., 1968).

2nd Stage:

$$\text{Succinylmonocholine} \xrightarrow[\substack{\text{Specific liver}\\ \text{enzymes + serum}\\ \text{cholinesterase}}]{\text{(Slow)}} \text{Succinic acid} + \text{choline}$$

A specific enzyme for the hydrolysis of succinyl monocholine is present (Greenway and Quastel, 1955), though a minor role is undertaken by serum cholinesterase also. Succinylmonocholine has 1/20th to 1/80th the activity of suxamethonium. Foldes et al. (1954) demonstrated that 5-7 mg./kg. of succinylmonocholine produced good relaxation in the anaesthetised patient for 8-12 minutes. These findings were confirmed by Brennan (1956).

In the absence of serum cholinesterase, alkaline hydrolysis of suxamethonium can occur. Kalow (1959) found that less than 5 percent per hour of suxamethonium is thus destroed. Normally this process plays a very minor role and less than 50 % of the injected drug is broken down in 10 hours.

The other processes which may play an important role in the absence of enzymatic hydrolysis are redistribution and excretion. Uptake of suxamethonium at various receptor sites reduces the drug concentration in the plasma (redistribution). The normally low levels of the drug excreted in urine may increase to much higher amounts in the absence of enzymatic hydrolysis (Wylie and Churchill-Davidson, A practice of Anaesthesia, 1978).

CHOLINESTERASE

Cholinesterases are the enzymes which hydrolyse the choline esters into the corresponding acid and free choline.

It was observed in 1914 by Sir Henry Dale that acetyl choline had a potent and short - lived action. He

suggested that an esterase destroys this compound in the human body.

Stedman, Stedman and Esson (1932) called this enzyme 'cholinesterase' and showed that it was of a specific nature.

Several biochemical differences in the cholinesterases of the R.B.C. and the serum of man were noticed by Alles and Hawes (1940). These two forms of the enzyme were named true cholinesterase and pseudocholinesterase by Mendel and Rudney (1943).

True Cholinesterase - This enzyme, also known as the "specific" or "aceto" or "e-type" cholinesterase is present in the red blood cells, neuromuscular junction and the brain. The Enzyme Commission has termed this enzyme acetylcholine acetylhydrolase with a code number E.C.3.1.1.7 . Its main function lies in hydrolysing acetyl choline rapidly. True cholinesterase also hydrolyses certain other choline esters such as beta-methyl-acetylecholine. It does not hydrolyse suxamethonium.

Serum cholinesterase - This enzyme is also known as "pseudocholinesterase" or "butyryl cholinesterase" or "non-specific cholinesterase" or "S-type cholinesterase". It has been found in the plasma, in most other tissues of the body like liver, kidney, intestine, pancreas etc. - but not in human erythrocytes. Its role shall be discussed later.

Nomenclature:

The modern name given to serum cholinesterase by the Enzyme Commission is acylcholine acylhydrolase (A.C.A.H.) with a code number E.C.3.1.1.8. (Dixon and Webb, 1974). The individual digits of this code-number express detailed information about the enzyme :

3 - stands for hydrolase (the general division).

1 - states the nature of bond hydrolysed -CHOH.

1 - means NADP (type of energy bond involved).

8 - denotes serial number of serum cholinesterase.

This classification also appears as such in the Enzyme Nomenclature Recommendations (1978).

The Second International meeting on Genetics in 1963 suggested some points regarding the terminology of cholinesterase. These were -

E - denotes the gene for serum cholinesterase. Subscript 1 stands for the first locus.

E_1^u - signifies gene responsible for formation of usual cholinesterase.

E_1^a - indicates the gene responsible for the formation of the atypical enzyme A.

E_1^s - implies the gene responsible for the absence of the enzyme (or as Whittaker, 1980 states - it denotes the gene responsible for an enzyme incapable of hydrolysing the cholinester bond).

E_1^f - denotes the gene responsible for the fluoride-resistant enzyme.

Assuming there are four allelic genes, there are ten genotypes - $E_1^u E_1^u$, $E_1^a E_1^a$, $E_1^s E_1^s$, $E_1^f E_1^f$, $E_1^u E_1^a$, $E_1^u E_1^s$, $E_1^u E_1^f$, $E_1^a E_1^f$, $E_1^a E_1^s$, $E_1^f E_1^s$.

Site of Production:

Serum cholinesterase, like albumin, is synthesized in the liver. Experiments on rats by Brauer and Root (1946) and by Ellis et al. (1947) demonstrated lowered serum cholinesterase following liver damage by carbon tetrachloride. This suggested that the enzyme was produced in the liver.

Experiment with carbon tetrachloride induced hepatic damage in dogs by Steensholt and Venndt (1945) had earlier showed a drop in serum albumin and rise of serum cholinesterase. Identical findings of an initial rise of the enzyme by Brauer and Root (1946) were attributed to outpouring of serum cholinesterase from liver during hepatic damage followed by a fall due to deficient production. These facts were brought out as early as 1946 in a compact from in the Transactions of the Fifth Conference on Liver Injury. Later work by Wescoe et al. (1947), Kaufman (1954) and Richterich (1961) proved beyond doubt that serum cholinesterase was produced in the liver. Histochemical evidence provided by Gurtner et al. (1963), further established that serum cholinesterase was produced only by functioning liver cells.

Physiological Role :

The physiological role of serum cholinesterase is still obscure. Lehmann and Silk (1953) suggest that it "protects" the "true" enzyme against many choline esters formed during metabolism which would otherwise inhibit the latter, by splitting them. Clitherow et al. (1963) agree with this theory. They propose that the main physiological function of serum cholinesterase may be to hydrolyze the butyryl-choline preferentially, so as to prevent its powerful nicotinic action, when it is produced during fatty acid degeneration. Thus Clitherow et al. (1963) suggest that serum cholinesterase is involved in lipid metabolism.

Others suggest that serum cholinesterase plays an essential role in transmission of slow nerve conduction processes (Bergmann and Wurzel, 1954), or that it functions in the removal of non-neurogenic acetyl choline, associated with spontaneous rhythmicity, and, at least in some animals, it is associated with the destruction of nervously - released acetyl choline also (Jamieson, 1963) which in supraoptimal concentration may inhibit the acetyl cholinesterase. Funnell and Oliver (1965) proposed that serum cholinesterase plays a regulatory role, in conjunction with choline acetylase in choline homeostasis in plasma.

Inspite of intermittent proposals regarding role of the enzyme it must be clarified here that no unequivocal

role has yet been assigned to serum cholinesterase.

Pharmacological Role :

Evidence of the pharmacological role of serum cholinesterase in hydrolysing suxamethonium was obtained when Bovet-Nitti demonstrated this in 1949.

Serum cholinesterase can also hydrolyse a large number of other choline esters like Benzoyl choline, phenylacetyl choline, Atrolacetyl choline, (Augustinsson, 1948) and some non-choline esters such as Tributyrin.

The local anaesthetic esters procaine and amethocaine owe their hydrolysis to serum cholinesterase. The hydrolysis of neostigmine also involves the enzyme intimately (Nowell Scott and Wilson, 1962).

Half Life:

The half life of cholinesterase has been estimated by measuring the fall of enzymic activity following the transfusion of plasma or injection of purified cholinesterase into an anenzymic patient and the various results of different workers are shown in the following concise table (Whittaker, 1980).

Contd. .....

Estimated half life of plasma cholinesterase.

Half life	Method
16 days	DFP
10 days	Plasma transfusion of anenzymic patient.
3 - 4 days	Plasma transfusion of anenzymic patient.
12 days	Purified ChE therapy of anenzymic patient.
8 - 9 days	Purified ChE therapy of anenzymic patient.
8 days	Purified ChE therapy of anenzymic patient.
44.7 h.	Purified ChE therapy of anenzymic patient.

'ChE' stands for plasma cholinesterase.

Whittaker (1980) regards the short half life reported in the last work (by Schuh, 1977) as very surprising due to the remarkable stability of the enzyme. Whittaker (1980) is of the opinion that a half-life in the range of 8 - 12 days seems probable.

Stability:

No appreciable variations in the cholinesterase activity of a given individual measured at irregular intervals over a period of 5 years were found by Wetstone and LaMotta (1965) in a study of 82 healthy adults. Lanks and Sklar (1976) found no significant change in enzyme activity in random specimens of whole blood stored at 4°C for 30 days but this procedure is not recommended due to frequently occurring haemolysis. Levels in plasma anticoagulated with heparin or EDTA declined only slightly. Therefore massive transfusions do not contraindicate suxamethonium administration. In separated plasma or serum, the enzyme remained stable for several weeks when stored at 0 - 5°C

(Witter, 1963). Epstein (1980) found that plasma frozen for 7 weeks at 70°C showed no decrease of enzyme activity. In the same study it is also reported that in bank blood stored at 4°C, of the total drop of enzyme activity 88 % occurred during the first 2 days of storage.

Johnston (1965) reports an approximately 30 % decrease of cholinesterase activity resulting from a single freezing and thawing of plasma. Whittaker (1980) however is of the opinion that plasma or serum may be stored at -20°C for several years without an appreciable loss of activity provided repeated freezing and thawing are not present. She further states that outdated plasma from National Blood Banks can be an useful source of large scale cholinesterase studies.

Interesting to note here is the observation of Pribilla (1957) that cholinesterase activity in plasma separated from autopsy blood upto 72 hours after death did not differ appreciably from the activity of samples from live subjects.

Physical and Chemical Properties:

A highly purified concentrated preparation of the serum cholinesterase is now available (Doenicke et al., 1968 and Goedde and Atland, 1971).

Serum cholinesterase is a glycoprotein with a molecular weight of about 300,000 (Surgener and Ellis, 1954)

This large molecule is composed of four polypeptide chains, each of molecular weight of about 80,000.

Serum cholinesterase migrates between alpha -2 and beta globulins in conventional paper electrophoresis. Svensmark (1961) has shown the presence of sialic acid, a mannose derivative. This is split off on incubation of serum with sialidase, leaving the same chemical activity as before but a gamma globulin like electrophoretic mobility. Svensmark (1961) suggests the attachment of several sialic acid molecules to each enzyme molecule at the non-active sites.

Starch-gel electrophoresis has resulted in the suggestion that serum cholinesterase may be heterogenous (Dubbs, Vivonia and Hilburn, 1960).

Wilson (1954) suggests that each cholinesterase molecule has two active sites which combine with one substrate molecule. The first of these - the esteratic site is presumed to be responsible for substrate hydrolysis by combining with it at the ester linkage. The second or anionic site is thought to be the negatively charged region of the enzyme surface which combines with the positively charged nitrogen atom in the choline radical of the substrate.

Unlike acetyl cholinesterase the serum cholinesterase has not been crystallised.

Methods of Estimation:

A variety of techniques have been employed by workers to estimate the values of serum cholinesterase. On the basis of these a list of the methods employed has been drawn up:

(1) The acid liberated can be titrated as it forms by a standard solution of an alkali (Kaufman, 1954).

(2) The acid liberated can be allowed to react with bicarbonate and the amount of CO_2 produced measured manometrically (McArdle, 1940).

(3) The rate of change of pH with time may be measured (Johnson and Whitehead, 1965).

(4) Measurement of the color change of indicators resulting from the lowering of pH of a weak buffer solution by the acid produced (Biggs, et al., 1958 and Steinitz et al. modification of Rappaport, et al. method, 1963).

(5) Measurement of acetyl choline ester remaining by using the hydroxamic acid reaction (de la Huerga's modification of Hestrin's method, 1952).

(6) Measurement of the amount of phenol liberated from a phenyl benzoate substrate (Smith, et al., 1959).

(7) Histochemical assay by the thiol analysis of acetyl thiocholine (Koelle and Friedenwald, 1949).

The Steinitz et al. modification (1963) of Rappaport et al. method, which has been used in this study has the advantage that large number of sera can be easily tested resulting in a screen test. Exact dibucaine and fluoride numbers can be obtained.

Differing ways of reporting the velocity of enzyme reactions tend to cause confusion. The amount of substrate transformed has arbitrarily been expressed in terms of milligrams, moles, equivalent and gas volumes or the reaction has been related to such changes in the reaction media as pH, absorbance, viscosity or turbidity. The manner in which time has been expressed has ranged from seconds to hours, while the reaction conditions have varied with respect to substrate, temperature, pH, buffer (type and molarity), ionic strength, co-factor concentrations and sample size (Bowers and McComb, 1970). The confusion arising from the use of arbitrary units composed of a multiplicity of variables has been vividly demonstrated for serum cholinesterase by Wetstone and La Motta (1965).

Results obtained by the different methods are not, in general, directly comparable because of the many differences of test-conditions descrived above. It is, therefore, necessary to compare results on a relative basis (Michel, 1961).

An 'Acholest' test strip method for detection of patients with low plasma cholinesterase is available, though

it is now considered that this method is inadequate (Dietz, 1972).

Normal Levels:

Normal values of serum cholinesterase by different methods are :-

Sl.No.	Method	Values (units/ml. of serum
1.	McArdle (1940)	60 - 120
2.	de La Huerga, et al. (1952)	150 - 305
3.	Biggs et al. (1958)	90 - 150
4.	Lehmann (1962)	60 - 120
5-	Steinitz et al. (1963)	60 - 100
6.	Johnson and Whitehead (1965)	207 - 403

Figures quoted here are for normal healthy adults.

A McArdle unit is defined as the amount of enzyme which will produce 1 microlitre of carbon dioxide by hydrolysis of acetyl choline in a bicarbonate buffer. The units of different workers' methods are different and the name of the worker whose method has been employed is usually given alongwith the values of cholinesterase mentioned.

Cholinesterase activity in healthy individuals:

Conflicting results regarding the influence of age and sex on the enzyme activity of normal healthy adults have been reported. No influence of age or sex was observed by Hall and Lucas (1937). Similar conclusions were drawn by

Callaway et al. (1951) in a series of 247 adults and by Vorhaus and Kark (1953). On the contrary, other workers such as Kalow and Gunn (1959), Wetstone and La Motta (1965) and Propert and Brackenridge (1976) are of the opinion that adult males have a higher cholinesterase activity than females.

At birth the enzyme activity is low (Lehmann et al., 1957) - it compared with about 50 % of the non-pregnant adult level (Zsigmond and Downs, 1971). The latter stated that this remains so until 6 months of age. In contrast, earlier workers like McCance et al. (1949) mentioned a dramatic rise to a greater - than - adult level in the first 3 weeks followed by maintenance till 3 years of age.

In the 3 to 6 years of age group Dabew (1970) found a mean cholinesterase activity of about 30 % above the adult level - this started decreasing during the fifth year to reach the adult level at puberty.

In adult level Kalow and Gunn (1959) reprted a negative correlation of enzymic activity with age, while in direct contradiction to this a positive correlation was reported by Propert and Brackenridge (1976).

No correlation between the serum cholinesterase levels and physical activity, diet, heart rate and blood pressure was noticed by Hall and Lucas (1937). During muscular exercise, Croft and Richter (1943) observed the rise of serum cholinesterase; Stoner and Wilson (1943) could not corroborate

this. No relation of enzyme level to weight, height or surface-area could be demonstrated by Vorhaus and Kark (1953).

Quantitative deficiency of Serum Cholinesterase :

It has already been pointed out that the deficiency of serum cholinesterase may be of a quantitative or a qualitative nature. In either of these conditions, the patients' ability to metabolise suxamethonium is impaired.

Among the first workers to study the variations of serum cholinesterase activity in different physiological and pathological conditions were Hall and Lucas (1937). Others who followed them were legion. The type of disorders causing a reduction in serum cholinesterase activity cover a broad spectrum.

That serum cholinesterase is produced in the liver has already been discussed. Antopol et al. (1938) first observed an invariable depression of the enzyme activity when hepatic parenchyma was diseased. Their finding was supported by Wescoe et al. (1947), Kunkel and Ward (1947) and Kaufman (1954). Vorhaus and Kark (1953) stated that on the whole, depression of the enzyme activity is more marked in patients ill with chronic liver disease such as cirrhosis, than in patients suffering from acute conditions. McArdle's (1940) finding were in agreement with the above general contention. A normal activity was noted in obstructive jaundice by McArdle (1940), Molander et al. (1954), Moore et al. (1957)

and many others. McArdle (1940) recorded depression of the enzyme activity in patients with tumours metastatic in the liver.

The relationship between serum albumin and cholinesterase levels was first observed by Faber (1943). Wetstone et al. (1960) reported a significant correlation (38 %) between the enzyme activity and serum albumin. Kunkel and Ward (1947) established that the only condition in which hypoalbuminaemia and a normal or high serum cholinesterase coexisted was the nephrotic syndrome.

Depression of the enzyme activity due to albumin transfusion was noticed by Vorhaus et al.(1950); an autoregulative mechanism for the control of these two was suggested to be responsible.

Value of serum cholinesterase estimation as a liver function test:

Although isolated serum cholinesterase estimations were proposed as an index of liver function by many workers (Vorhaus and Kark, 1953; Kaufman, 1954; Molander et al., 1954), this was not advocated by Whittaker (1980). The argument for the latter opinion is that due to the wide range of normal results obtained and the difficulty in comparing the results with those of various other investigators because of numerous differences in technique, isolated cholinesterase estimations are not advisable or of value as an index of liver function.

Further complicating the issue are a host of factors affecting serum cholinesterase activity; it may not be practically possible to eliminate all of these in subjects undergoing cholinesterase estimations for the purpose of assessing liver function. Serial estimations of the enzyme in the same patient, however, are of greater value in assessing the prognosis of disease and the recovery (Vorhaus and Kark, 1953; Kaufman, 1954; Hunt and Lehmann, 1960). Serial assays of the enzyme have been used by Hunt and Lehmann (1960) in determining the prognosis of venous shunt operations of the portal system.

Leyine and Hoyt (1949), Molander et al. (1954), Wetstone et al. (1960), Kaniaris et al. (1979) and Ghooi et al. (1980) found reduced serum cholinesterase activity in cases of malignancy. The reduction of activity was more in instances where the tumour had spread to other sites - specially to the liver (Ghooi et al., 1980). The carcinomas were associated with maximum reduction of the enzyme activity (Wetstone et al., 1960). The site of the primary growth had a bearing on the fall of the enzyme levels. Maximum reduction was seen when the primary sites were lung, gastrointestinal tract and genitourinary tract - carcinoma of the lung being associated with most markedly reduced enzyme levels (Kaniaris et al., 1979). The cause of this malignancy - associated reduction of enzyme activity was not primarily the hepatic parenchymal involvement due to either tumour cell invasion or prolonged extrahepatic

biliary obstruction. Carcinomatous tissue per se was responsible (Wetstone et al., 1960); this was presumed to be responsible for production of a serum cholinesterase inhibitor (Kaniaris et al., 1979).

Depressed serum cholinesterase levels were also reported in cases with malnutrition by Milhorat (1938), Faber (1943) and Vorhaus and Kark (1953). Their findings were supported by the work of Waterlow (1950) and Barclay (1973). A high incidence (83 %) of low enzyme levels was discovered in a study of 302 malnutrition cases by Barclay (1973). Almas and Prathapkumar (1969) showed decreased levels in cases of kwashiorkor with vitamin A deficiency. Khalil (1980) reported lowered levels in 3 patients of crohn's disease.

It was demonstrated by Milhorat (1938), Sawitsky (1949) and Scudamore et al. (1951) that low levels of serum cholinesterase existed in various forms of chronic anaemias and blood dyscrasias. Abnormal levels have, however, never been found in patients ill with sickle cell anaemia (Sawitsky, 1949) or hypoplastic anaemias or polycythemia (Vorhaus and Kark, 1953). Scudamore et al. (1951) found that the serum cholinesterase level increased slowly, parallel to the improvement in haematological and clinical status of the patient.

Levine and Hoyt (1949) could not demonstrate any decrease in the level of serum cholinesterase in patients with

pulmonary tuverculosis. This was in contrast to the earlier results of Jones and Stadie (1939) who had found low levels in far advanced tuberculosis and carcinoma. McArdle (1940) found decreased activity in uraemia. Reduced levels were reported in uraemia and shock (Editorial, British Medical Journal, 1951).

Surgical shock was found to share a place among the factors causing a depression of the enzyme activity (Doenicke and Holle, 1962). This depression has been attributed to the surgical intervention itself rather than the anaesthetic agents since patients undergoing only ophthalmic, rather than general surgery showed no change in the enzyme activity.

It was observed by Hall and Lucas (1937) that low serum cholinesterase values were frequently found to be associated with acute infections. The converse was not always true. Antopol et al. (1937) and Vorhaus and Kark (1953) substantiated these findings. Hodges and Harkness (1954) noticed that low enzyme levels were most characteristically associated with "ill", debilitated patients in a condition which was easier, perhaps, to recognise than to define. Hyperpyrexia has also been found to be associated with low cholinesterase levels (Antopol et al., 1937).

Therapeutic radiation has been reported to be associated with low enzyme levels by Hodges and Harkness

(1954). Low levels have been seen to occur after dialysis with the Kolff twin-coil kidney (Holmes et al., 1958) and in patients on haemodialysis (Thomas and Holmes, 1970)

In the latter instance, the workers have suggested the patient's renal failure rather than the dialysis to be the responsible factor. Low values were seen in patients who underwent plasmapheresis by Wood and Hall (1978) and Evans et al. (1980). A single 4 liter, plasma exchange can reduce cholinesterase by 64 % and repeated daily plasma exchanges can virtually remove cholinesterase altogether.

Exposure or poisoning by a variety of organophosphorous compounds has been reported in man by Barnes and Davies (1951). Phosphorylation of the active enzyme site by the organophosphorous compound permits accumulation of the acetyl choline at the nerve ending and patients present symptoms typical of cholinergic poisoning in such cases.

Unlike the unanimous opinion regarding depressed cholinesterase levels in patients of liver and biliary diseases, the view regarding the enzyme levels during pregnancy and the post-partum period is divided. Although Hall and Lucas (1937) and Meade and Rosalski (1963) found no correlation between enzyme levels and pregnancy or early puerperium, the majority evidence favours the presence of a decreased cholinesterase activity during the above periods.

This reduction of serum cholinesterase activity has

been found to commence after the 10th week of pregnancy (Robertson, 1966). In agreement are Hazel and Monier (1971), Blitt et al. (1977) and Evans and Wroe (1980), who state that the fall of enzyme activity starts during the first trimester of pregnancy. This reduction of enzyme activity, which is maintained during the rest of pregnancy, has been reported by various workers, to be of the order of 25 % (Levine and Hoyt, 1049), 21 % (Pritchard, 1955), 28 % (Shnider, 1965), 18 % (Robertson, 1966), 21 % (Hazel and Monier, 1971) and 30 % (Redderson, 1973).

Post partum levels of the enzyme have been found to be depressed further. Hazel and Monier (1971) and Blitt et al. (1977) found a 33 % and 32 % reduction respectively of the enzyme level on the 3rd day post partum. The gradual return of the enzyme level to normal occurs between the tenth day to seventh week post partum (Wildsmith, 1972).

Pritchard (1955) attributed this fall of enzyme activity to haemodilution. Robertson (1966) later suggested that the incriminating factors might be haemodilution altered hepatic function, anti-cholinesterase effects of oestrogen and malnutrition present in these patients. The reduction of enzyme activity has been found to be less marked in patients with toxaemia by Pritchard (1955) and Robertson (1966).

A higher proportion of heterozygotes would be expected to show sensitivity to suxamethonium during pregnancy than when non-pregnant according to the lower cholinesterase

characteristics of the heterozygote.

Tetanus has been assigned a place among the conditions resulting in a reduction of serum cholinesterase (Porath et al. 1977). Earlier, Werle and Stuttgen (1942) had noted the cholinesterase depressing effect of tetanotoxin, which was found to be nearly equal to that of organophophorous compounds. Hanna et al. (1979), however, noted an increased cholinesterase activity in 3 out of 6 tetanus patients and observed that this increase was related to the severity of the disease.

Moore et al. (1957) and Sharma and Seth (1978) discovered low levels of serum cholinesterase in myocardial infarction. It was suggested that a persistently falling enzyme level was seen in fatal cases. Reduction in enzyme levels was seen in 50 cases of acute head injury by Rao et al. (1978), who also stated the prognostic value of the estimation and found that - significantly low levels implied poor prognosis. Bush et al. (1962) found reduced values in severly burned patients and it has since been suggested that the concurrent hepatic lesion of burns is the main culprit here. Myxoedematous patients were seen to possess serum cholinesterase activity upto 30 % lower than normal (Thompson and Whittaker, 1965).

Rheumatoid arthritis (Milstor, 1970) and polyarteritis nodosa (Potts and Thornton, 1961) have been seen to be associated with low enzyme values. In polyarteritis nodosa the decrease has been blamed upon the more than frequently recognised liver

involvement. It has been stated that all muscle relaxants should be avoided or used with extreme caution in the presence of polymyositis, dermatomyositis, systemic lupus erythematosus and polyarteritis nodosa, unless prior investigations of hepatic function have been found to be normal.

Another cause of reduction of enzyme activity is the use of the anticancer drug AB - 132 (Wang and Ross, 1963) where a dose related effect was observed. Reduced levels are also seen due to the anticholinesterase drugs neostigmine, physostigmine, pyridostigmine, ecothiopate iodide, edrophonium, hexafluorenium (Kopman et al., 1978) and due Ketamine hydrochloride, fluothane, ethrane (Carrasco et al. 1978). Pancuronium (Stovner et al., 1975).

Propanidid and trimethaphan have also been implicated in their association with drugs causing reduced serum cholinesterase levels. Oral contraceptives containing oestrogen cause a decreased cholinesterase activity (Robertson, 1967) and also a modification of cholinesterase isoenzymes (Bergmann and Warzel, 1954). These changes are reversible by withdrawal of the contraceptive.

Quantitatively increased levels of serum cholinesterase:

There were conditions in which quantitatively increased levels of serum cholinesterase were observed. Berry et al. (1954) observed higher than normal levels in "fat" individuals in a study of 354 cases. A highly significant

positive correlation was found by them between surface fat and serum cholinesterase. Later Thompson and Trounce (1956) attributed the high cholinesterase levels seen sometime in diabetes to the associated obesity and not to the disease itself.

An increase of serum cholinesterase to about 20 % above the normal level was seen in thyrotoxicosis patients by Thompson and Whittaker (1965). High values have also been observed in asthma and alcoholism by Vaccarezza and Peltz (1960), nephrosis by Kunkel and Ward (1947), essential hypertension by Vorhaus (1952) and in psoriasis, nodular goitre and hyperlipaemia. In certain mental disorders like anxiety or depressive states (Tod and Jones, 1937) and schizophrenia (Tod and Jones, 1937; Antebi and King, 1962; Propert, 1979) the serum cholinesterase levels have been foun to be increased. Propert (1979) did not find the increased levels to be of significance.

Relationship between low serum cholinesterase levels and suxamethonium apnoea:

Various workers have put forward differing opinions regarding the presence or absence of any relationship between serum cholinesterase activity and the duration of action of suxamethonium.

Lehmann and Ryan (1956) reported 27 cases of prolonged apnoea. Out of these 6 patients had normal and the

remaining 21 low enzyme levels. Argent et al. (1955) found 1 patient with low enzyme level in a study of 7 cases of prolonged apnoea, Kalow (1959) found that approximetely 10 % of his cases of prolonged apnoea were due to very low enzyme activity.

Bourne et al. (1952) noted lower mean cholinesterase levels in patients who showed delayed recovery, in their study of 546 cases:

	Mean ± Standard Error (units of Callaway et al.)
Delayed recovery -	38.3 ± 6.8 units/ml.
Normal recovery -	88.5 ± 6.9 units/ml.

Foldes et al. (1956) demonstrated that the decreased levels seen in association with liver disorders did not usually cause more than a three fold increase in the duration of apnoea.

Lievre (1980) in a study of 280 randomly selected surgical patients found that only 11 (3.9 %) had levels below the normal range; of these only 1 developed prolonged apnoea. It was concluded therefore that a low cholinesterase level is not an infallible indicator of post-surgical complications.

Blitt et al. (1977) found no correlation between the enzyme activity and duration of paralysis when they administered a 40-80 mg. per body surface area dose of suxamethonium.

In contrast, Kalow and Gunn (1957) had found the Logarithmic relationship between dose and response to be linear,

with normal distribution, of the logarithms of the dose required to produce a standard effect and the logarithms of the duration of apnoea after standard doses.

Reduction of the enzymatic activity to less than 25 % by Vickers (1963) and less than 50 % by Whittaker (1980) has been felt necessary before any significant prolongation of the suxamethonium is observed.

As a generalisation, however, the lower the cholinesterase activity and the dibucaine and fluoride numbers, the more prolonged is the response to succinyl choline. The failure of consistency of this relationship is due to technically incorrect estimations. A valid assay of cholinesterase can only be carried out at 37°C - such an assay shows good correlation between cholinesterase activity and patient sensitivity (McLaren and Moffit, 1976).

Genetic variants of the enzyme:

Prolonged apnoea in response to the administration of suxamethonium was noticed by many earlier workers (Bourne, et al., 1952; Argent et al. 1955). Forbat (1953) first postulated the idea that the result of an inherited defect in the enzyme metabolism. Lehmann and Ryan (1956) suggested that the genetic defect was transmitted as an autosomal recessive trait. Over the period 1957 - 1960, Kalow and his associates worked to demonstrate a qualitative difference between the altered enzyme and the normal one. Kalow (1956) had earlier identified the former enzyme and termed it "atypical" esterase, since it

demonstrated a reduced affinity for and hydrolysis rate of choline ester substrates such as suxamethonium.

Kalow and Genest (1957) demonstrated that the atypical cholinesterase could be detected by its inhibition with varying concentrations of a suitable inhibitor such as the local analgesic drug dibucaine hyrochloride (Cinchocaine or Nupercaine). Kalow and Genest (1957) introduced the term dibucaine number (D.N.). This was defined as the percentage inhibition by a fixed concentration of dibucaine (10^{-5} M) of the rate of hydrolysis of benzoylcholine under standard conditions of temperature, pH, buffer and fixed concentration of 10^{-5} M dibucaine is routinely used to determine dibucaine numbers in different sera.

Dibucaine nuber in patients very sensitive to suxamethonium is approximately 20, while in normal patients is around 80. An intermediate group with a dibucaine number of about 60 is also found. The table below shows the dibucaine numbers of various types of sera:

	Genetic status	Dibucaine number	Population in group
A. Normal homozygote	$E_1^u E_1^u$	70 - 85	96.2 %
B. Heterozygote	$E_1^u E_1^a$	50 - 65	3.8 %
C. Abnormal homozygote	$E_1^a E_1^a$	16 - 25	1 : 2800

(Wylie and Churchill-Davidson, A practice of Anaesthesia, 1978).

The biosynthesis of serum cholinesterase is controlled by two allelic genes, E_1^u and E_1^a (Kalow and Staron, 1957), and

these are a non-dominant autosomal in nature. Only one gene can exist at locus 1 on each of the chromosome of a pair. Each gene controls the production of its enzyme.

Separation of two types of enzymes from presumed heterozygotes by Lidell et al. (1962) confirmed that there were actually two physically different entities. Only one enzyme could be separated from homozygotes using paper electrophoresis or column chromatography using anion exchange material diethyl aminoethyl cellulose.

A knowledge of the dibucaine number can help in predicting the genetic configuration of the patient.

The advantage of dibucaine number determination is that a clear trimodal distribution indicating three distinct genotypes is present which is totally tacking in the Gaussian distribution of activity (Whittaker, 1980).

The dibucaine number of any person changes only after transfusion of blood from an individual with a different genotype (Whittaker, 1980).

It is regarded that the presence of atypical cholinesterase is the main and more serious cause of suxamethonium apnoea of prolonged duration (Editorial, Lancet, 1973).

Whittaker (1980) feels that a higher proportion of heterozygotes would be expected to show sensitivity to suxamethonium during pregnancy than when non-pregnant. This is due to the low cholinesterase characteristics of the heterozygotes $E_1^u E_1^a$.

It is significant to mention here that the ethnic group lacking the E_1^a gene, like Zimbabwe - Rhodesia Africans are very susceptible to leprosy (Whittaker et al., 1976).

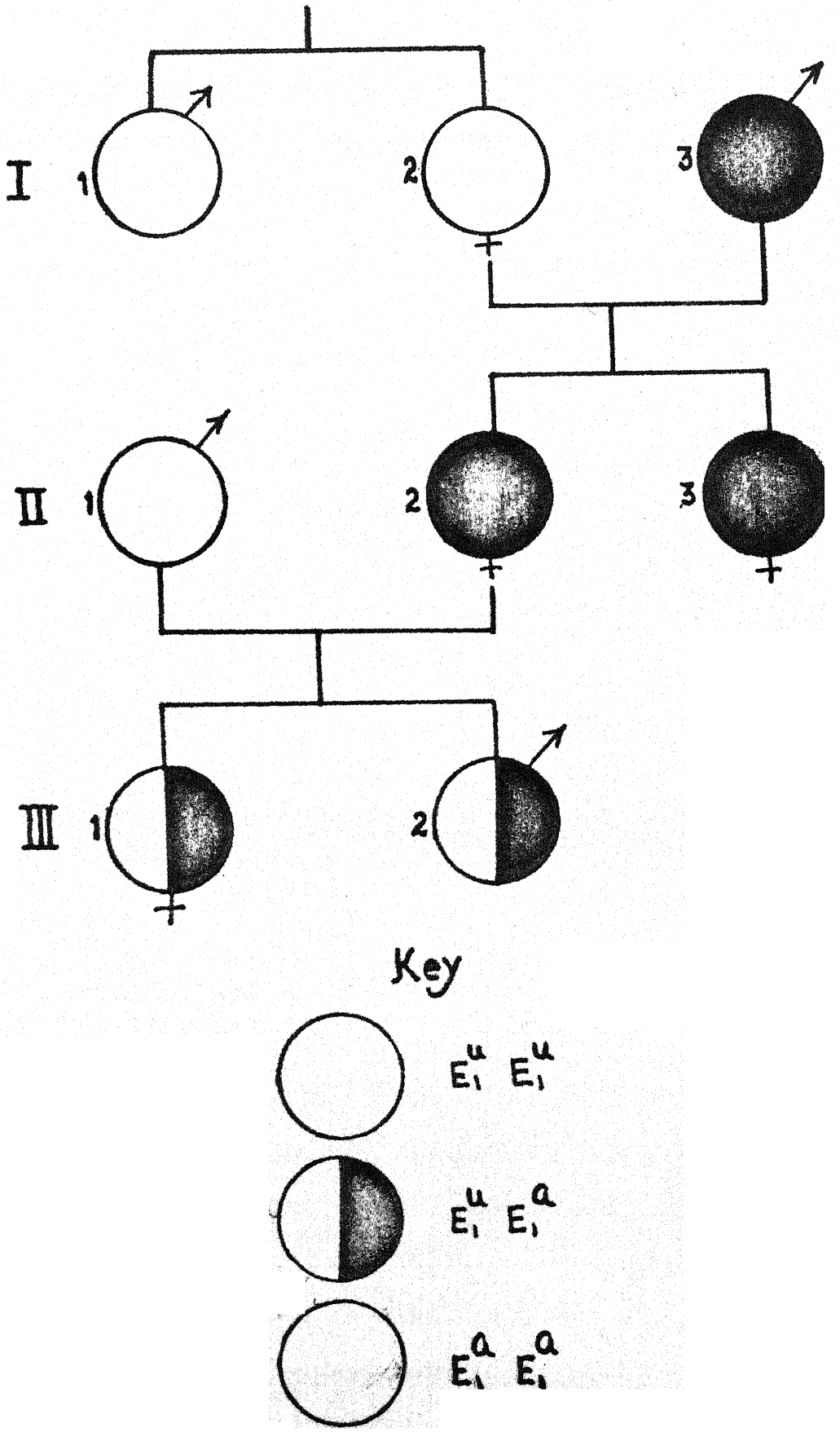
Lubin et al. (1971) found a heterozygote male preponderance among Caucasians of 1.85 : 1 in a preschool nutrition survey. They suggested the possibility of a sex modification and environmental influence on this polymorphic system. They were of the opinion that the atypical allele was presumably absent in Japanese, Eskimoes and South American Indians and was rare in Negroes, Australian aboriginees, Filipinos and Oriental populations (other than Japanese)

Kalow and Gunn (1957) observed that heterozygotes to atypical plasma cholinesterase do not have greatly prolonged responses to succinyl choline.

Silent gene:

The discovery of this and the events leading to it are interesting. In case of an atypical homozygote (E_1^a E_1^a) for simple Mendelian recessive inheritance, both parents must have at least one E_1^a gene and moreover all the children of such an individual should have an E_1^a gene. But in a family in Kalow and Staron's (1957) study, such inheritance patterns were not seen (Fig.3). The presence of a silent - gene was then proposed. Whittaker (1980) suggests that this gene is not really 'silent' since it does not imply non-production of the gene, but the biosynthesis of cholinesterase lacking

Fig. 3 - PEDIGREE OF FAMILY WITH UNUSUAL INHERITANCE AS DETERMINED BY DIBUCAINE NUMBERS.



the structure required to hydrolyse the choline-ester bond. Thus no enzymic activity is present.

Applying this concept to the individuals in Fig. 3 I_2 should be a heterozygote $E_1^u E_1^s$ (D.N. - 80) while her children II_2 and II_3 become heterozygotes $E_1^a E_1^s$ (D.N. - 20).

Later Lidell and his colleagues (1962) reported the presence of silent-gene with no enzymatic activity ($E_1^s E_1^s$). Many such anenzymic individuals have since been described. In some ethnic groups like Alaskan Eskimoes, some Caucasian South Africans there is a large incidence of the silent gene. Vysas of Andhra Pradesh have also been seen to have a high incidence of the silent allele (Rao, 1979).

Two different types of silent gene have been recognised (Scott and Wright, 1976). One type of cholinesterase deficiency is definitely anenzymic with no activity; the other type has 2 - 8 % of the normal mean cholinesterase activity. Scott and Wright (1976) point towards at least two or three genetic defects in the enzyme which may be present. The probability of other silent gene variants leaves this field open to further research (Whittaker, 1980).

While homozygotes for E_1^s gene are recognised due to little or no enzyme activity, the detection of the E_1^s heterozygote is very difficult without extensive family studies. The reason for this is that the inhibition reactions of homozygotes of other variants (like $E_1^a E_1^a$) and the heterozygote of that variant and silent gene (like $E_1^a E_1^s$) are similar.

Fluoride-resistant gene:

Another gene controlling the cholinesterase biosynthesis is the fluoride-resistant gene E_1^f . It was discovered by Harris and Whittaker (1961) by using sodium fluoride to differentially inhibit it. The fluoride number (F.N.) is the percentage inhibition by a fixed concentration (5×10^{-5} M) of sodium fluoride of the rate of hydrolysis of benzoyl choline under standard conditions of temperature, pH, buffer and fixed concentration of substrate. The dependancy of fluoride-inhibition on temperature must be stressed here.

A high frequency of the E_1^f gene has been found in psychoses and Huttington's disease (Whittaker and Berry, 1977).

Other rare variants:

Other variants of cholinesterase at the E_1-locus have been suggested. Whittaker (1960) reported the presence of a chloride-resistant gene.

Two other rare genes - E_1^j (Rubinstein et al., 1976) in one family and E_1^k (Ruvinstein et al., 1978) in two family have been found.

Another gene associated with a very high cholinesterase activity has been described by Nietlich (1966). It is known as the E. Cynthiana variant or the Nietlich variant. Its locus of functioning is not known. However, It has been suggested that mutation of normal or silent gene to an abnormal or rare one may occur at times (Evans and Magill, 1974).

An electrophoretic variant (C_{5+}) was discovered by Harris et al. (1963). It functions at the cholinesterase locus E_2 and is genetically determined due to an autosomal dominant gene. Persons having this variant have a high cholinesterase activity but Whittaker (1980) does not consider it to be of importance to the anaesthetist.

Identification of the cholinesterase variants:

A variety of inhibitors have been used. These include sodium chloride, urea, sodium bromide, succinyl-dicholine, RO2 - 0683 the dimethyl carbamate of (2-hydroxy-5-phenyl-benzyl) trimethylammonium bromide, formaldehyde, thyroxine and alkyl alcohols. These inhibitors have been used with either choline esters or non-choline esters as substrates (Whittaker, 1980).

Whittaker (1980) states that though such numerous and diverse systems can be daunting, as new techniques become available, the number of recognised cholinesterase variants will increase.

Screening programmes for cholinesterase variants:

Genetically important relatives of individuals of individuals known to be sensitive to suxamethonium should be investigated for cholinesterase variants (Whittaker, 1980). There is no significant morbidity or mortality directly attributable to succinyl choline apnoea which requires to be balanced against a limited success rate for generalised screening of all patients about to receive suxamethonium (McLaren and Moffit,

1976).

Lievre (1980) states that programmes concerning aberrations occurring in 1 - 10 % frequency are feasible; those occurring in less than 1 % frequency are not feasible. The frequency of homozygotes is less than 0.1 % but heterozygotes occur in 3.8 % frequency. This puts abnormal cholinesterase levels into the range of feasible genetic screening programmes.

The problem of increased sensitivity to suxamethonium is not purely genetic, but on the basis of those at comparable risk due to acquired abnormality seems justifiable (McLaren and Moffit, 1976; Lievre, 1980; Whittaker, 1980).

Some causes of increased plasma cholinesterase activity

Type	Condition
Inherited	Electrophoretic variants C_{5+}
	Nietlich or Cynthiana variant
Acquired	Obesity
	Hyperlipaemia
	Nodular goiter
	Psoriasis
	Essential hypertension
	Thyrotoxicosis
	Nephrosis
	Asthma
	Anxiety states
	Alcoholism
	Schizophrenia

(Whittaker, M., 1980).

Some causes of decreased plasma cholinesterase activity

Type	Condition
Inherited deficiencies	Rare cholinesterase variants
Physiologicalvariance	Last trimester of pregnancy
	New borns and infants
Acquired causes	Liver diseases (acute hepatitis and hepatic metastasis)
	Myocardial infarction
	Collagen diseases (progressive muscular dystrophy, congenital myotonia, dermatomyositis)
	Hyperpyrexia
	Tuberculosis
	Acute infections
	Carcinomas
	Chronic debilitating diseases
	Surgical shock
	Chronic anaemias
	Uraemia
	Malnutrition
	Myxoedema
Iatrogenic causes	X-ray therapy
	Anti-cancer drugs
	Monoamine oxidase inhibitors
	Contraceptive pills
	Ecothiopate iodide
	Propanidid
	Neostigmine
	Chlorpromazine chloride
	Pancuronium
	Organophosphorus insecticides
	Burned patients

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Type	Condition
	Cyclophosphamide
	Extracorporeal circulation
	Rheumatic fever
	Typhus
	Tetanus
	Kwashiorkor
	Epilepsy

(Whittaker, M., 1980).

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Distribution, suxamethonium sensitivity and biochemical characteristics of the plasma cholinesterase variants in a British population

Genotype	Relative mean enzymic activity	Dibucaine number Mean	Dibucaine number Range	Fluoride number Mean	Fluoride number Range	Frequency	Suxamethonium sensitivity
$E_1^u E_1^u$	100	80	77–83	61	56–56	96 %	? 1 in 2500 moderately sensitive
$E_1^u E_1^s$	50	80	77–83	61	56–68	1 in 190	? 1 in 1000 moderately sensitive
$E_1^u E_1^f$	86	74	70–83	52	46–54	1 in 200	? 1 in 100 moderately sensitive
$E_1^u E_1^a$	77	62	48–69	50	44–54	1 in 25	? 1 in 500 moderately sensitive
$E_1^a E_1^f$	59	53	45–59	33	28–39	1 in 20,000	All moderately sensitive
$E^f{}_1 E_1^f$	74	67	64–69	36	34–43	1 in 154,000	All moderately sinsitive
$E_1^f E_1^s$	37	67	64–69	36	34–43	1 in 150,000	All moderately sensitive
$E^a{}_1 E_1^a$	43	21	8–28	19	10–28	1 in 2000	All very sensitive
$E_1^a E_1^s$	22	21	8–28	19	10–28	1 in 29000	All very sensitive
$E_1^s E_1^s$	Enzymic activity nil or too low to measure					1 in 100,000	All very sensitive

(Whittaker, M., 1980).

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MATERIAL AND METHODS

M A T E R I A L A N D M E T H O D S

This study was conducted on patients admitted to the M.L.B. Medical College and Hospital, Jhansi, from August, 1981 to February, 1982. The cases were chosen from patients undergoing various surgical procedures under General Anaesthesia.

SELECTION OF CASES:

Apart from the normal healthy subjects chosen as control group, the selection of other cases was done on the basis of some factors known to influence serum cholinesterase level. A total No. of 93 cases belonging to both sexes and various age groups from (0-10) years to(61-70) years were divided into :-

1. Control Group : This constituted of 26 normal healthy individuals with no apparent factor likely to have any bearing on the serum cholinesterase level.

2. Anaemia and Malnutrition : This included 21 individuals with haemoglobin levels less than 10 gm. % or with a history of marked weight loss and evident by their asthenic to cachexic look. These patients often complained of listlessness, easy fatiguability and were prey to frequent infections of various types. Borderline cases of malnutrition were not included in this study.

3. Pregnancy : This comprised of 15 women who underwent Caesarean section or medical termination of pregnancy.

Fig. 4 - SHOWING THE DISTRIBUTION OF POPULATION INTO DIFFERENT STUDY GROUPS.

4. Post-Partum : Six patients in early puerperium who underwent abdominal ligations composed this group.

5. Dehydration : This group contained a total of nine patients with acute abdominal conditions having clinically evident dehydration.

6. Malignancy : This group comprised of four patients with malignancies proved on histopathological examination.

7. Liver and Biliary diseases : A group of three patients with liver dysfunction proved by albumin levels, less than 3 gm. % (normal 3.5 to 5.5 gm. %) and abnormal liver function tests.

8. Mental disorder : Making up this group were three patients of diagnosed mental disorder who underwent electroconvulsive therapy or some other necessary operation.

9. Thyroid disorder : Assembled in this group were four patients of thyroid conditions such as thyroid adenoma, goitre and mild thyrotoxicosis.

10. Obesity : This group had two patients both grossly overweight in respect to their frame and height.

11. Children: The group consisted of six normal healthy children between 10 and 14 years of age.

Due to the presence of multiple factors in some (7) patients, they were placed separately under more than one group.

The patients were classified into high, high middle, low middle and low socioeconomic groups on the basis of a criteria prepared for the purpose (Appendix II).

The patients were also grouped according to their place of residence (Fig. 5).

PREOPERATIVE ASSESSMENT :-

The patients included in the study were clinically examined preoperatively before any premedication was administered. Details were recorded on a proforma prepared for the purpose (Appendix I).

SAMPLE COLLECTION :-

5 ml. blood was withdrawn before operation into a clean dry test tube. As soon as clot retraction and serum separation had occurred, the serum was withdrawn and kept in a plain vial which was maintained in a deep freezer at -20°C till needed for estimations.

PREMEDICATION :-

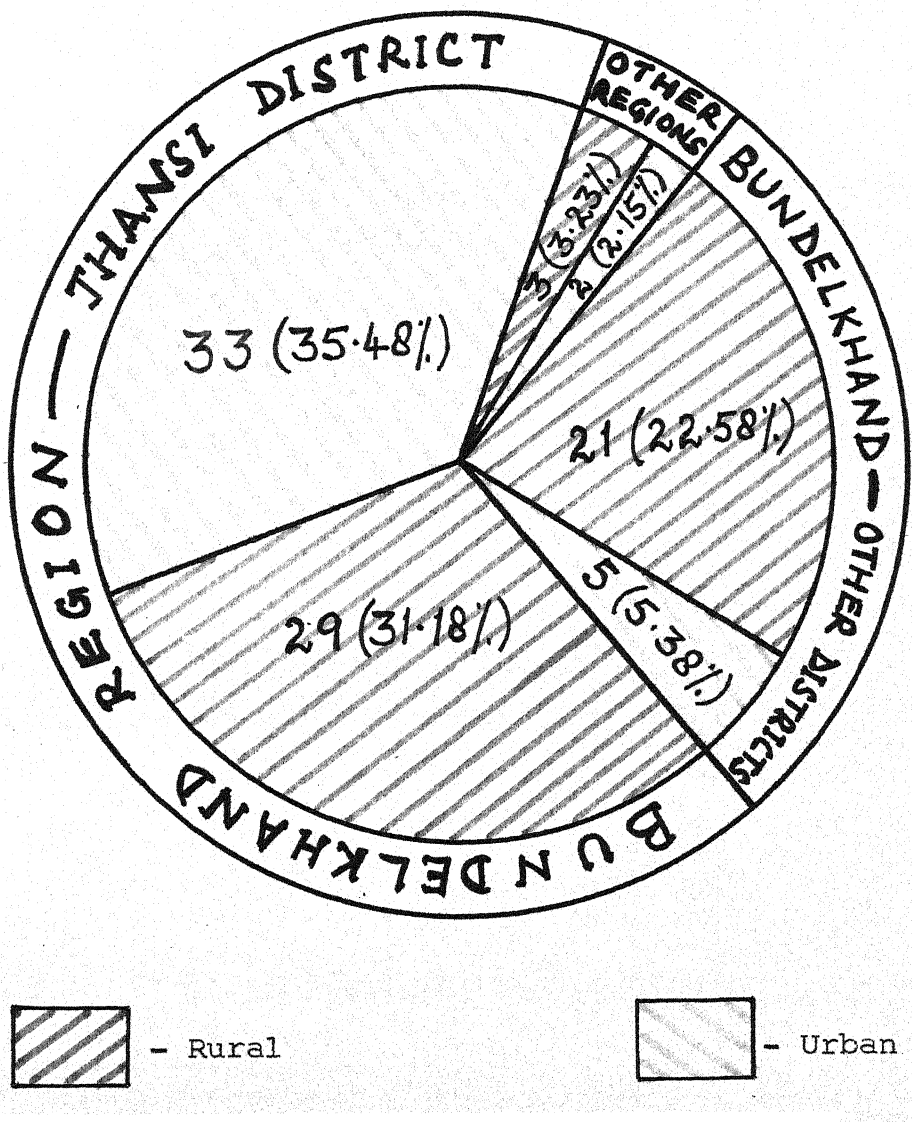
Patients were premedicated with 0.01 mg./kg. of body weight of Atropine. Narcotic analgesics were not used for premedication to avoid any influence on the duration of apnoea.

ANAESTHESIA :

The patients were induced with sleep dose of 2.5 % intravenous (I.V.) thiopentone, followed immediately by a 1 mg./kg. I.V. dose of suxamethonium. The stopwatch was switched on to record the start of suxamethonium apnoea as heralded by disappearance of reservoir bag movement.

Patients were oxygenated with mask and intermittent positive pressure ventilation (I.P.P.V.) followed by direct vision intubation and connection to the Boyle's apparatus.

Fig. 5 - PIE CHART SHOWING RESIDENTIAL DISTRIBUTION OF THE STUDY POPULATION.



The chest was auscultated. The patient was subsequently maintained with oxygen and nitrous oxide at the rate of 12-16 min.. The I.P.P.V. was withheld every 1/2 minute to catch the first return of respiratory effort as noted by reservoir bag movement. The stopwatch was then switched off.

METHOD OF SERUM CHOLINESTERASE ESTIMATION :

The serum cholinesterase was estimated by the Steinitz et al. modification (1963) of Rappaport et al. method (1959). This method is based on the decolourisation of meta-nitrophenol by the acetic acid freed during the action of cholinesterase on acetyl choline substrate. This decrease in colour is a measure of the acetic acid formed and hence of the enzyme activity. For "atypical" and "fluoride-resistant" cholinesterases additional substrates containing dibucaine for the former and sodium fluoride for the latter were used.

REAGENTS :- (1) Buffer - (a) 6.65 gm. anhydrous disodium phosphate (Na_2HPO_4) and 0.43 gm. of monopotassium phophate (KH_2PO_4) were dissolved in about 200 ml. of distilled water. (b) 0.30 gm. of m-nitrophenol was dissolved in about 200 ml. of distilled water while heating it slightly. After mixing (a) and (b) and adjusting pH to 7.8 by the addition of 0.1 N NaOH, the volume was made upto 1000 ml. with distilled water. The colorimetric reading of this solution was about 300 in the Klett Summerson colorimeter. If it was higher, dilution with the same buffer (6.65 gm. Na_2HPO_4 and 0.43 gm. KH_2PO_4 made upto 1 L. and adjusted to pH 7.8) was done.

(2) Acetyl choline chloride or bromide (15 %) (kept in refrigerator) - 0.1 ml. in 3.5 ml. final volume resulted in approximately 2 x 10^{-2} M solution.

(3) 0.9 % Nacl.

(4) 0.1 N Acetic Acid.

(5) (a) Dibucaine (mol. wt. 380). Stock solution of 44.3 mg. in 100 ml. distilled water.

(b) Stock solution was diluted 10 times before use (0.3 ml. in 3.5 ml. final volume resulting in 10^{-3} M concentration).

(6) Sodium Fluoride - 210 mg./L.. This resulted in a 5 x 10^{-3} M solution.

(7) Neostigmine - 1 ampoules of 0.5 mg./ml.).

PROCEDURE :-

Three test tube designated T (test), D (dibucaine) and F (fluoride) were used for each determination. Two additional test tubes, S (standard) and B (blank) were used for each seried of determination. For all above determinations haemolysed or turbid serum was not used, specially so in case of the Standard and Blank tests where in addition to above the serum was not icteric also.

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To the test tubes T, D, F, S and B were added reagents in the order and amounts shown in the table below :

Reagent	T (ml.)	D (ml.)	F (ml.)	Standard (ml.)	Blank (ml.)
* Serum	0.1	0.1	0.1	0.1	0.1
* Buffer	3.0	3.0	3.0	3.0	3.0
* 0.9 % Nacl	0.3	-	-	0.2	0.3
* Neostigmine	-	-	-	0.1	0.1
* 0.1 N Acetic acid (0.1 ml. corresponds tp 100 units)	-	-	-	0.1	-
* Dibucaine (diluted)	-	0.3	-	-	-
* Sodium Fluoride	-	-	0.3	-	-
* Acetyl choline	0.1	0.1	0.1	0.1	0.1
WATER - BATH - - - 25°C, 30 min.					
* Neostigmine	0.1	0.1	0.1	-	-

Results were read on the Klett-Summerson colorimeter. The absorbance was read at 420 millimicrons (filter 42), setting to zero absorbance with water.

For values of cholinesterase above 96 units/ml., the serum was diluted with an equal volume of the sodium chloride solution and the test was repeated taking 0.1 ml. of diluted serum and multiplying the result by 2.

CALCULATIONS :-

(1) $$\text{Cholinesterase units} = \frac{\text{Standard units}}{B - St} \times (B - T)$$

(2) $$\text{Activity after Dibucaine} = \frac{\text{Standard units}}{B - St} \times (B - D)$$

(3) $$\text{Dibucaine Number} = 100 - \frac{100 \times \text{Dibucaine Activity}}{\text{Cholinesterase units}}$$

(4) $$\text{Activity after Fluoride} = \frac{\text{Standard units}}{B - St} \times (B - F)$$

(5) $$\text{Fluoride Number} = 100 - \frac{100 \times \text{Fluoride Activity}}{\text{Cholinesterase units}}$$

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OBSERVATIONS & RESULTS

O B S E R V A T I O N S

This study of the relationship between suxamethonium apnoea and serum cholinesterase in the local population was carried out on 93 patients admitted to indoor wards at the M.L.B. Medical College and Hospital, Jhansi from August 1981 to February 1982. The patients were from both sexes and age-groups from (0-10) years to (61-70) years.

These patients were divided into 26 normal healthy individuals (control group) and 21 cases of anaemia and malnutrition, 15 cases of pregnancy, 6 cases in the post-partum period, 9 patients with dehydration, 4 cases of malignancy, 3 cases of liver and biliary diseases, 3 cases of mental disorder, 4 cases of thyroid disorder, 2 cases of obesity and 6 normal healthy children, the latter being taken to observe the variations - if any - in the paediatric age group. 1 case who was otherwise normal except for the unduly prolonged suxamethonium apnoea was also included. Each of the seven patients who had presence of multiple factors was placed in more than one group accordingly.

After completion of the study following observations have been made:

Table 1 shows the normal healthy individuals or control group consisting of 16 males and 10 females. It shows serum cholinesterase activity ranging from 62.0 units/ml. to

98.3 units/ml.. The mean enzyme value is 77.84 units/ml.. Mean Dibucaine Number (D.N.) is 78.38; mean Fluoride Number (F.N.) is 59.62. Duration of apnoea ranged from 3 to 10 minutes with a mean time of 4.69 minutes. In addition, on obeerving D.N. and F.N. of the patients, Case No. 6 appears to be a heterozygote for the fluoride-resistant and Case No. 16 a heterozygote for the atypical gene.

Table 1 - CONTROL GROUP (Normal Healthy Individuals)

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	2	30	F	73.6	74	66	6
2.	* 6	25	M	68.0	78	49	9
3.	7	50	M	78.0	83	59	5
4.	10	17	F	77.4	79	61	4
5.	11	32	M	86.1	89	56	4
6.	*16	22	M	63.6	56	50	10
7.	23	18	M	78.0	86	63	3
8.	28	32	M	72.6	76	55	4
9.	32	40	F	62.0	79	59	6
10.	33	43	F	86.4	78	60	4
11.	34	18	F	73.6	78	57	4
12.	37	43	M	81.6	88	59	4
13.	40	18	M	85.0	77	64	3
14.	42	43	M	69.0	84	67	6
15.	45	65	M	88.3	73	61	7
16.	51	40	M	75.0	75	62	3
17.	62	34	F	87.2	82	60	3
18.	64	29	M	76.8	87	59	6
19.	71	27	M	80.9	82	57	4
20.	73	57	M	98.3	72	66	3
21.	75	26	M	78.5	76	55	5

Contd.....

Table 1 contd. .....

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
22.	81	25	F	83.4	75	62	4
23.	83	20	F	73.1	78	58	5
24.	84	18	F	70.7	70	65	3
25.	87	60	F	77.8	80	59	4
26.	90	30	M	79.0	83	61	3
Range				62.0-98.3	56-89	49-67	3-10
Mean ± S.D.				77.84±8.10	78.38	59.62	4.69
Standard Error				1.5885	-	-	-

'ChE' stands for serum cholinesterase. '*' stands for a heterozygote.
'S.D.' stands for standard deviation.

Table 2 - Anaemia and Malnutrition.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	8	40	F	40.0	82	62	6
2.	12	56	M	61.6	83	57	5
3.	*21	40	M	44.6	59	42	9
4.	22	68	M	52.0	79	58	7
5.	25	70	M	47.3	77	59	5
6.	26	40	F	43.4	81	60	6
7.	30	18	F	60.1	76	58	5
8.	35	60	F	57.4	76	59	5
9.	38	40	F	45.2	74	63	7
10.	48	25	F	68.0	76	63	3
11.	49	21	F	57.8	79	66	6
12.	59	26	M	48.2	72	63	7
13.	*60	22	F	44.8	70	51	6
14.	66	22	F	30.0	75	68	15
15.	69	50	F	46.6	85	64	5
16.	72	26	F	41.4	78	62	3

Contd. .....

Table 2 contd. .....

6

Sl. No.	Case No.	Age in years	Sex	ChE-units/ml.	D.N.	F.N.	Period of apnoea (minutes)
17.	76	22	M	50.6	81	57	8
18.	80	70	F	48.7	84	69	6
19.	85	34	M	59.4	84	60	8
20.	91	22	F	48.7	76	66	6
21.	*93	25	F	36.2	59	42	25
Range				30.0-68.0	59-85	42-69	3-25
Mean ± S.D.				49.14±9.13	76.48	59.48	7.29
Standard Error				1.992	-	-	-

The above table (Table 2) is a summary of cases with anaemia and malnutrition. It shows the presence of two atypical heterozygotes - Case numbers 21 and 93. A fluoride-resistant heterozygote is Case No. 60. Cholinesterase levels ranging from 30.0 units/ml. to 68.0 units/ml., with a mean of 49.14 units/ml. are seen, showing a highly significant difference from the control group. The mean duration of apnoea is 7.29 minutes.

The following tables (Table 3 and 4) show mean cholinesterase levels of 57.77 units/ml. and 50.75 units/ml. in pregnancy and postpartum cases respectively. The differences of enzyme levels between each of these groups and the control group are statistically highly significant, though the difference between either of these is not significant.

Table 3 - Pregnancy.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	* 1	28	F	67.4	58	46	9
2.	4	22	F	50.3	78	61	8
3.	14	28	F	64.3	72	63	5
4.	15	28	F	60.6	80	65	3
5.	17	32	F	68.6	77	59	5
6.	26	40	F	43.4	81	60	6
7.	29	30	F	68.4	74	62	6
8.	39	32	F	59.4	70	59	3
9.	43	20	F	69.0	80	60	5
10.	48	25	F	68.0	76	63	3
11.	57	26	F	58.8	89	60	4
12.	66	22	F	30.0	75	68	15
13.	70	26	F	66.0	81	66	4
14.	86	18	F	56.2	76	63	4
15.	*93	25	F	36.2	59	42	25
Range				30.0-69.0	58-89	42-68	3-25
Mean ± S.D.				57.77±12.47	75.07	59.8	7
Standard Error				3.2197	-	-	-

Mean ChE shows 25.78 % reduction from control group.

Table 4 - Post-Partum.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	27	30	F	40.1	74	61	6
2.	36	26	F	50.1	79	58	4
3.	41	30	F	46.0	75	59	3
4.	47	28	F	43.2	83	61	6
5.	58	28	F	58.4	80	56	4
6.	65	25	F	66.7	76	57	3

Contd. ....

Table 4 contd. ....

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
Range				40.1-66.7	74-83	56-61	3-6
Mean ± S.D.				50.75±10.06	77.83	58.67	4.33
Standard Error				4.1070	-	-	-

Mean ChE shows 34.80 % reduction from control group.

Compared with the non-pregnant adults (controls) pregnancy cases showed a 25.78 % reduction of the mean cholinesterase activity; in case of post-partum patients this reduction was 34.80 %. Case numbers 1 and 93 were atypical heterozygotes as suggested by their D.N. and F.N..

The following table (Table 5) shows the highly significant difference of mean cholinesterase value in cases of dehydration (59.94 unites/ml.) as compared to that of the control group. Mean duration of action of suxamethonium is 9.22 minutes. Contributing to this is a 35 min. apnoea in case No. 3 - which was an atypical heterozygote (in addition to case No. 21).

Table 5 - Dehydration.

Sl. No.	Case No.	Age in years	Sex	ChE units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	* 3	35	M	50.5	44	49	35
2.	13	55	F	76.4	76	56	4
3.	19	25	M	78.0	81	62	4
4.	20	23	M	55.2	85	61	7

Contd. ...

Table 5 contd. ....

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
5.	*21	40	M	44.6	59	42	9
6.	25	70	M	47.3	77	59	5
7.	52	45	F	59.6	78	63	6
8.	63	58	M	61.1	75	58	7
9.	67	24	M	66.8	78	57	6
Range				44.6-78.0	44-85	42-63	4-35
Mean ± S.D.				59.94±12.0	72.56	56.3	9.22
Standard Error				4.0000	-	-	-

Table 6 - Malignancy.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	9	23	M	63.0	80	63	6
2.	35	60	F	57.4	76	59	5
3.	46	40	F	40.6	73	57	8
4.	89	35	F	69.9	79	61	6
Range				40.6-69.9	73-80	57-63	5-8
Mean ± S.D.				57.73±12.51	77	60	6.25
Standard Error				6.255	-	-	-

Thus a mean enzyme level of 57.73 units/ml. which is highly significant when compared with controls is observed in the Malignancy-group. (Table 6) Average D.N. is 77 and average F.N. is 60 and mean duration of action of suxamethonium is 6.25 minutes.

In the following table of liver and biliary diseases (Table 7) a mean cholinesterase activity of 50.90 units/ml. is seen. Average value for D.N. is 79.3, F.N. is 59.7, duration of apnoea is 6.7 minutes. p-value is highly significant.

Table 7 - Liver and Biliary Diseases.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	18	40	M	41.6	76	63	6
2.	50	39	F	62.9	86	58	7
3.	74	30	F	48.2	76	58	7
Range				41.6-62.9	76-86	58-63	6-7
Mean ± S.D.				50.90±10.90	79.3	59.7	6.7
Standard Error				6.2931	-	-	-

Table 8 - Mental.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	5	22	M	81.4	79	60	2
2.	31	57	M	99.3	72	68	1
3.	54	20	F	76.7	78	57	4
Range				76.7-99.3	72-79	57-68	1-4
Mean ± S.D.				85.80±11.93	76.3	61.7	2.33
Standard Error				6.8878	-	-	-

The above (Table 8) shows a mean cholinesterase level of 85.8 units/ml. in mental cases. The difference of this from mean control level is not significant statistically. Average duration of suxamethonium apnoea was 2.33 minutes.

Table 9 shows higher than normal mean cholinesterase level in Thyroid cases (88.6 units/ml.). This difference is statistically significant.

Table 9 - Thyroid.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	53	50	F	106.3	88	61	2
2.	56	30	F	78.1	73	60	5
3.	88	15	F	95.4	79	64	3
4.	92	40	F	74.6	78	57	4
Range				74.6-106.3	73-88	57-64	2-5
Mean ± S.D.				88.60±14.90	79.5	60.5	3.5
Standard Error				7.45	-	-	-

Table 10 - Obesity.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	55	43	F	101.6	77	62	2
2.	68	49	F	112.3	81	58	1
Range				101.6-112.3	77-81	58-62	1-2
Mean ± S.D.				106.95±7.57	79	60	1.5
Standard Error				5.3528	-	-	-

Very high average serum cholinesterase activity (106.95 units/ml.) is seen in the obese patients (Table 10). The difference from controls is highly significant. Mean duration of action of suxamethonium is 1.5 minutes only.

The following table (Table 11) shows a slightly more than normal - but statistically insignificant - average value of the enzyme (80.67 units/ml.). Average duration of action of suxamethonium was 5.33 minutes.

Table 11 - Children.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	44	12	M	89.6	83	64	6
2.	61	10	M	80.2	81	62	6
3.	77	12	F	78.0	80	67	5
4.	78	10	F	79.4	78	63	4
5.	79	10	M	80.8	78	58	4
6.	82	12	M	76.0	77	59	7
Range				76.0-89.6	77-83	58-67	4-7
Mean ± S.D.				80.67±4.70	79.5	62.17	5.33
Standard Error				1.9188	-	-	-

Table 12 - Abnormal Homozygote.

Sl. No.	Case No.	Age in years	Sex	ChE-units/ ml.	D.N.	F.N.	Period of apnoea (minutes)
1.	24	35	M	27.2	18	30	40

Contd. ....

Table 13 - Statistical analysis of the serum cholinesterase levels in the various study groups

Sl. No.	Name of group	Population of group (n)	Mean ChE±S.D. (ChE in units/ ml.)	Statistical significance (as compared to control group)			
				t(cal.)	D.F.	p-value	significance
1.	Control	26	77.84±8.10	-	-	-	-
2.	Anaemia and Malnutrition	21	49.14±9.13	11.5261	45	$<.001$	Highly significant
3.	Pregnancy	15	57.77±12.47	6.3513	39	$<.001$	Highly significant
4.	Post-Partum	6	50.75±10.06	7.1102	30	$<.001$	Highly significant
5.	Dehydration	9	59.94±12.00	4.9861	33	$<.001$	Highly significant
6.	Malignancy	4	57.73±12.51	4.2904	28	$<.001$	Highly significant
7.	Liver and Biliary diseases	3	50.90±10.90	5.2927	27	$<.001$	Highly significant
8.	Mental	3	85.80±11.93	1.5456	27	$>.05$	Insignificant
9.	Thyroid	4	88.60±14.90	2.1959	28	$<.05$	Significant
10.	Obesity	2	106.95±7.57	4.9339	26	$<.001$	Highly significant
11.	Children	6	80.67±4.70	0.8227	30	$>.05$	Insignificant

Between Pregnancy and Post-Partum groups, t(cal.) = 1.2316; D.F. = 19; p $>.05$; Insignificant

Fig. 6 - SHOWING CHOLINESTERASE LEVELS AGAINST DURATION OF APNOEA IN EACH GROUP.

In order to study the influence of age, sex, residential-status, alcohol, diet, socioeconomic status and smoking on the serum cholinesterase levels the normal healthy individuals were chosen. This was done so as to prevent the concurrent influence on the enzyme level of any other factor known to affect it.

The following table (Table 14) shows the mean cholinesterase levels of various age groups from (0-10) years to (61-70) years. The results indicate presence of a slightly higher serum cholinesterase activity in children (upto 14 years of age), followed by a fall in level which is maintained till 50 years of age. Thereafter a rise in the activity is seen in patients of (51-60) years and (61-70) years age groups.

Contd. .....

Table 14 - Mean cholinesterase levels of various age groups : (Children + Control group)

Sl. No.	Age group (in years)	Mean age of group (in years)	Strength of group No.	%	Range of ChE value (units/ml.)	Mean ChE values (units/ml.)	Statistical significance t(cal.)	DF	p	Significance
1.	0-10	10.00	3	9.38	79.4-80.8	80.13	.4811	27	7.05	I
2.	11-14	12.33	3	9.38	76.0-89.6	81.20	.6843	27	7.05	I
3.	15-20	18.17	6	18.75	70.7-85.0	76.30	.4451	30	7.05	I
4.	21-30	26.75	8	25.00	63.6-83.4	75.48	.7352	32	7.05	I
5.	31-40	35.60	5	15.63	62.0-87.2	76.58	.3036	29	7.05	I
6.	41-50	44.75	4	12.50	69.0-86.4	78.75	.2102	28	7.05	I
7.	51-60	58.50	2	6.25	77.8-98.3	88.05	1.6575	26	7.05	I
8.	61-70	65.00	1	3.13	88.3	88.30	-	-	-	-

'I' stands for insignificant.

Table 15 - Control group : Mean cholinesterase levels in the two sexes.

Sl. No.	Sex	Mean age of group (in years)	Strength of group No.	%	Range of ChE value (units/ml.)	Mean ChE values (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	Significance
1.	Male	34.81	16	61.54	63.6-98.3	78.67	.3144	40	7.05	I
2.	Female	30.50	10	38.46	62.0-87.2	76.52	.4459	34	7.05	I

Between Male and Female : t(cal.)= .6555; D.F.= 24; p 7.05; Insignificant.

Thus mean cholinesterase activity of males (78.67 units/ml.) is slightly greater than that of females (76.52 units/ml.) though this difference is statistically insignificant.

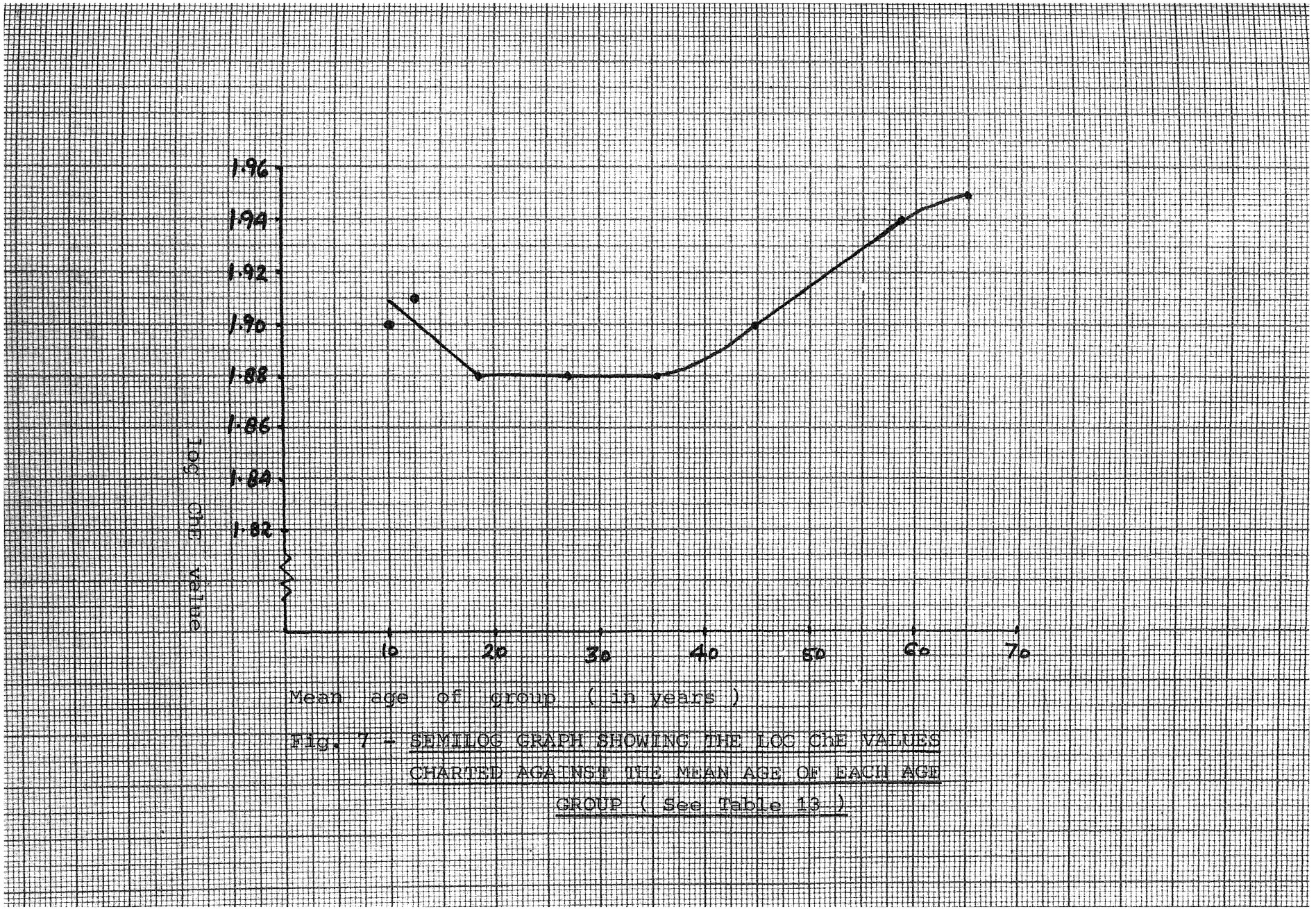


Fig. 7 - SEMILOG GRAPH SHOWING THE LOG ChE VALUES CHARTED AGAINST THE MEAN AGE OF EACH AGE GROUP (See Table 13)

Although slight variations in the serum cholinesterase activity in persons hailing from different residential backgrounds was noticed (Table 16), none of these were of any statistical significance.

Table 16 - Control group : Residential distribution v/s cholinesterase levels.

Sl. No.	Resident of	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	significance
1.	Other Regions	4	15.38	73.6-85.0	78.28	.1012	28	$>.05$	I
2.	Bundelkhand Region	22	84.62	62.0-98.3	77.76	.0332	46	$>.05$	I
(a)	Jhansi district	15	57.69	62.0-98.3	77.97	.0487	39	$>.05$	I
(b)	Other districts	7	26.92	63.6-88.3	77.33	.1433	31	$>.05$	I
(i)	Urban areas	14	53.85	62.0-87.2	76.87	.3716	38	$>.05$	I
(ii)	Rural areas	12	46.15	63.6-98.3	78.98	.3891	36	$>.05$	I

Between 1 and 2 : t(cal.)= .1143; D.F.= 24; p $>.05$; Insignificant.
Between 2(a) and 2(b) : t)cal.)= .1584; D.F.= 20; p $>.05$; Insignificant.
Between (i) and (ii) : t(cal.)= .6614; D.F.= 24; p $>.05$; Insignificant.

Table 17 - Control group : Alcohol users and non-users v/s cholinesterase levels.

Sl. No.	Group	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	significance
1.	Alcohol users	3	11.54	76.8-86.1	81.27	.7116	27	$>.05$	I
2.	Alcohol non-users	23	88.46	62.0-98.3	77.40	.1841	47	$>.05$	I

Between alcohol users and non-users : t(cal.)= .7771; D.F.= 24; p $>.05$; Insignificant.

Thus it is evident that alcohol users showed slightly higher enzyme levels than alcohol non-users (Table 17).

Table 18 - Control group : Vegetarian and non-vegetarian patients v/s cholinesterase levels.

Sl. No.	Group	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	Significance
1.	Vegetarians	15	57.69	62.0-98.3	78.53	.3142	35	7.05	I
2.	Non-vegetarians	11	42.31	63.6-88.3	76.91	.2695	39	7.05	I

Between vegetarians and non-vegetarians : t(cal.)= .5000; D.F.= 24; p 7.05; Insignificant.

It was seen that in vegetarian patients the cholinesterase levels were slightly higher than in the non-vegetarian patients (Table 18).

Smokers had greater average serum cholinesterase activity than non-smokers (Table 19). The enzyme activity in Bidi-smokers was more than in patients who smoked Cigarettes.

Table 19 - Control group : Smoking status v/s cholinesterase level.

Sl. No.	Smoking status	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	Significance
1.	Non-smokers	15	57.69	62.0-87.2	76.28	.6393	39	7.05	I
2.	Smokers	11	42.31	63.6-98.3	79.97	.6916	35	7.05	I
(a)	Bidi smokers	7	26.92	63.6-98.3	82.19	-	-	-	--
(b)	Cigarette smokers	4	15.38	68.0-81.6	76.10	-	-	-	-

Between smokers and non-smokers : t(cal.)= 1.1460; D.F.= 24; p 7.05; Insignificant.
Between Bidi and Cigarette smokers : t(cal.)= 1.0083; D.F.= 9; p 7.05; Insignificant.

The following table (Table 20) shows a greater enzyme activity in the low middle class (3) than in the low class (4). The high middle class (2) again shows a slight fall of

cholinesterase activity though this does not bear a great significance since this group is composed of 2 patients only.

Table 20 - Control group : Socioeconomic status v/s cholinesterase level.

Sl. No.	Socioeconomic status	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Statistical significance (compared with control group) t(cal.)	DF	p	Significance
1.	Low class (4)	12	46.15	62.0-98.3	76.24	.5300	36	7.05	I
2.	Low middle class (3)	12	46.15	68.0-87.2	79.78	.7266	36	7.05	I
3.	High middle class (2)	2	7.69	70.7-80.9	75.80	.3327	24	7.05	I
4.	High class (1)	0	0.00	-	-	-	-	-	-

Table 21 - Routine surgery and Emergency surgery patients v/s cholinesterase values and duration of apnoea.

Sl. No.	Nature of case	Strength of group No.	%	Range of ChE values (units/ml.)	Mean ChE value (units/ml.)	Range of duration of apnoea (minutes)	Mean of duration of apnoea (minutes)	Statistical significance (compared with Controls) t(cal.)	DF	p	Significance
1.	Routine	68	73.12	27.2-112.3	68.09	1-40	5.32	.6446	159	7.05	I
2.	Emergency	25	26.88	30.0-80.2	61.21	3-35	8.00	1.3065	115	7.05	I

Between Routine and Emergency cases : t(cal.)= 1.7286; D.F.= 91; p 7.05; Insignificant.

Patients undergoing routine surgical procedures were seen to have a greater enzyme activity than those undergoing emergency operations (Table 21). The former had a shorter duration of action of suxamethonium.

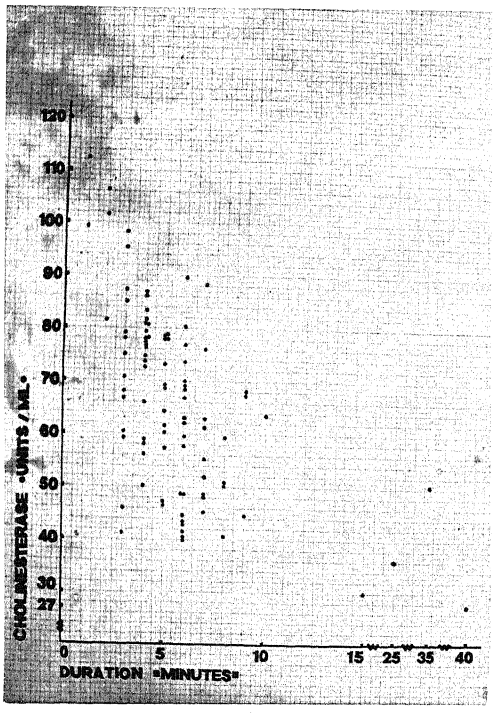


Fig. 8 - SCATTERGRAM SHOWING CHOLINESTERASE VALUE DISTRIBUTION AGAINST THE DURATION OF ACTION OF SUXAMETHONIUM.

Karl Pearson's Coefficient of correlation between cholinesterase and duration of apnoea (r_1) = - .4766392

A limited degree of negative correlation exists between ChE value and the duration of apnoea in this study. Thus, when ChE value falls, the duration of apnoea increases and vice-versa.

Regression line of y (ChE) on x (duration) :

The above is the 'line of average relationship' between serum cholinesterase values and duration of apnoea. It follows the regression equation :

ChE = 92.6 - 5.05 x duration.

Where,

ChE -is the serum cholinesterase value in units/ml..

Duration -is the duration of apnoea in minutes.

Through this equation we can have a rough idea of the duration of apnoea or the serum cholinesterase level in any patient in this study, provided any one of the above factors (ChE or Duration) is known.

' Sy ' is the standard error of the estimate.

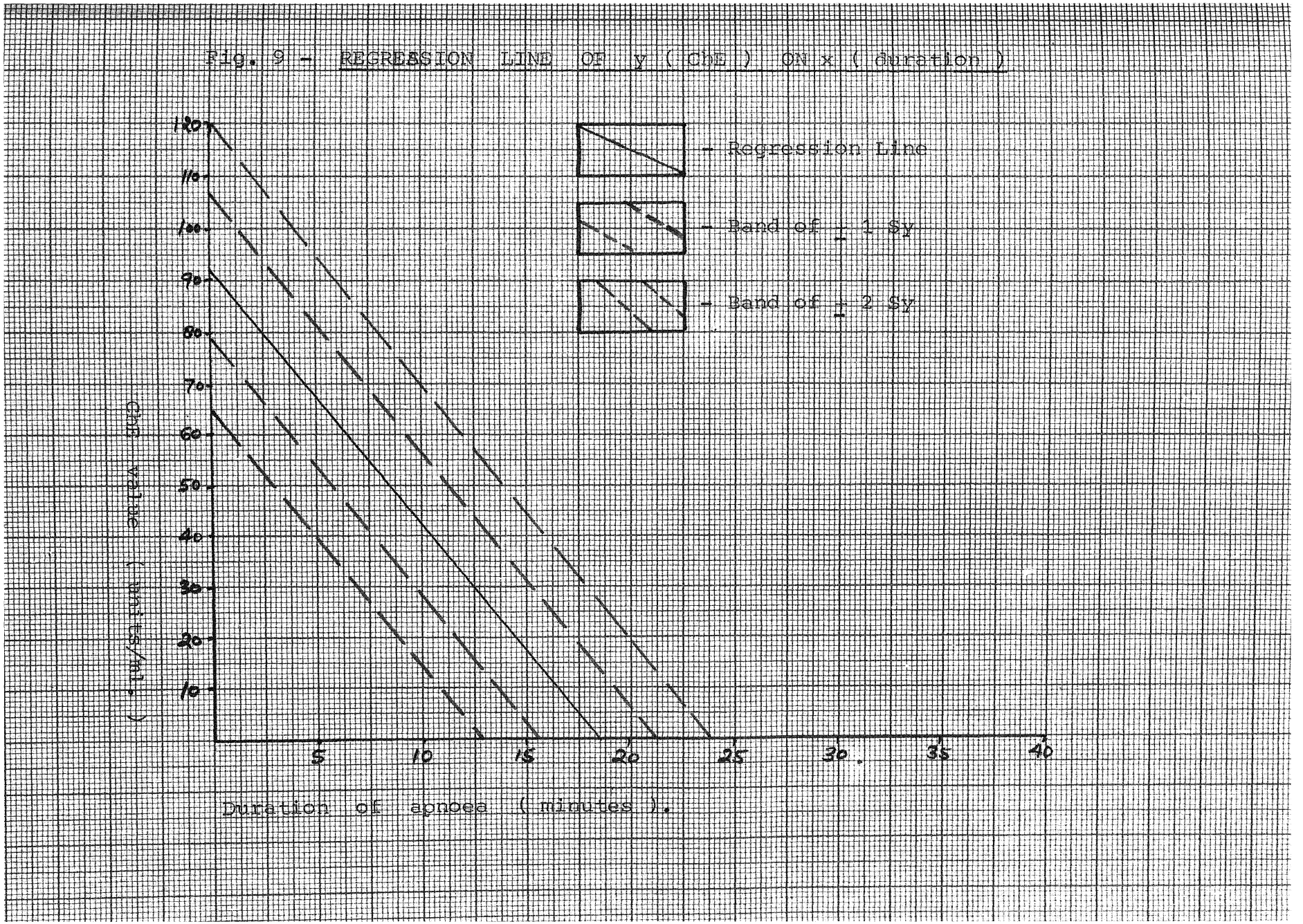
Here,

Sy = 13.52 .

The band formed by (ChE $\pm$ 1 Sy), shall have 68.27 % (approximately 2/3) of the points on the scatter diagram (Fig.9).

Similarly, the band formed by (ChE $\pm$ 2 Sy) shall have 95.45 % of the points on the scatter diagram (Fig.9).

Fig. 9 - REGRESSION LINE OF y (ChE) ON x (duration)



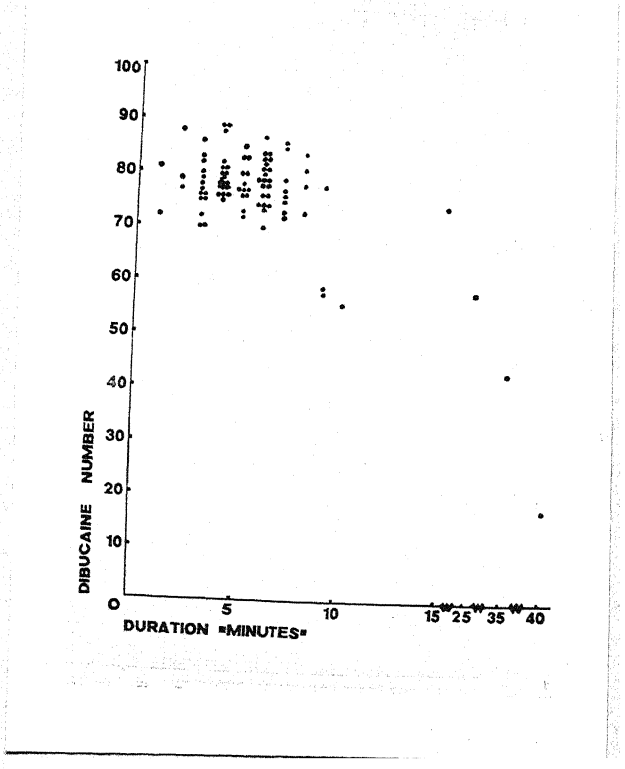


Fig. 10 - SCATTERGRAM SHOWING DIBUCAINE NUMBER DISTRIBUTION AGAINST THE DURATION OF ACTION OF SUXAMETHONIUM.

Karl Pearson's Coefficient of correlation between D.N. and duration of apnoea (r_2) = - .7152 .

A limited degree of negative correlation exists between D.N. and the duration of apnoea in this study. Thus, when D.N. value falls, the duration of apnoea increases and vice-versa.

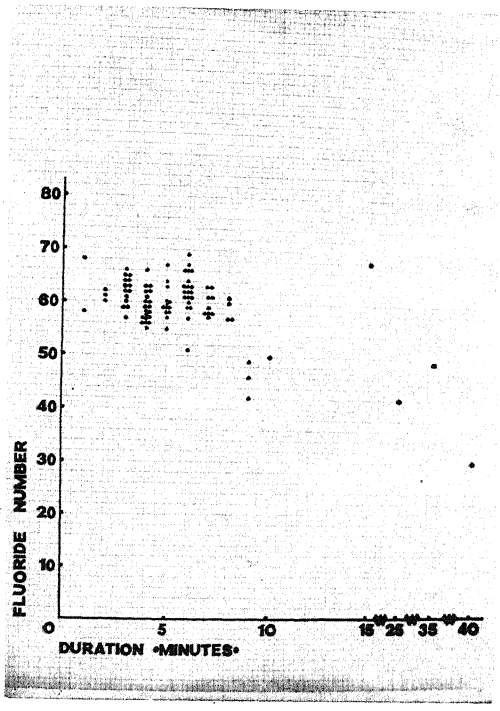


Fig. 11 - SCATTERGRAM SHOWING FLUORIDE NUMBER DISTRIBUTION AGAINST THE DURATION OF ACTION OF SUXAMETHONIUM.

Karl Pearson's Coefficient of correlation between F.N. and duration of apnoea (r_3) = - .6358

A limited degree of negative correlation exists between F.N. and the duration of apnoea in this study. Thus, when F.N. value falls, the duration of apnoea increases and vice-versa.

Table 22 - Distribution of genotypes in the study.

Sl. No.	Genotype	No. of cases	% Distribution of genotypes
1.	$E_1^u E_1^u$	85	91.40
2.	$E_1^u E_1^a$	5	5.38
3.	$E_1^u E_1^f$	2	2.15
4.	$E_1^a E_1^f$	0	0.00
5.	$E_1^a E_1^a$	1	1.08
6.	$E_1^s E_1^s$	0	0.00

Of the 93 total cases in the study, 85 cases were normal homozygotes ($E_1^u E_1^u$) and 1 was an abnormal homozygote ($E_1^a E_1^a$) (Table 22). The 7 heterozygotes present in this study included 5 cases heterozygous for the usual and atypical gene ($E_1^u E_1^a$) and 2 heterozygotes of the usual and fluoride - resistant genes ($E_1^u E_1^f$) (Fig.12).

Table 23 shows that the maximum number of cases, 37 (39.78%) had a duration of action of suxamethonium (period of apnoea) between 2 - 4 minutes, followed by 33 cases (35.48 %) in the 5 - 6 minutes range. Patients showing a period of apnoea between 11 - 15 minutes and 16 - 30 minutes numbered 1 (1.08 %) each (Fig.13).

Fig. 12 - SHOWING THE DISTRIBUTION OF GENOTYPES IN THE STUDY.

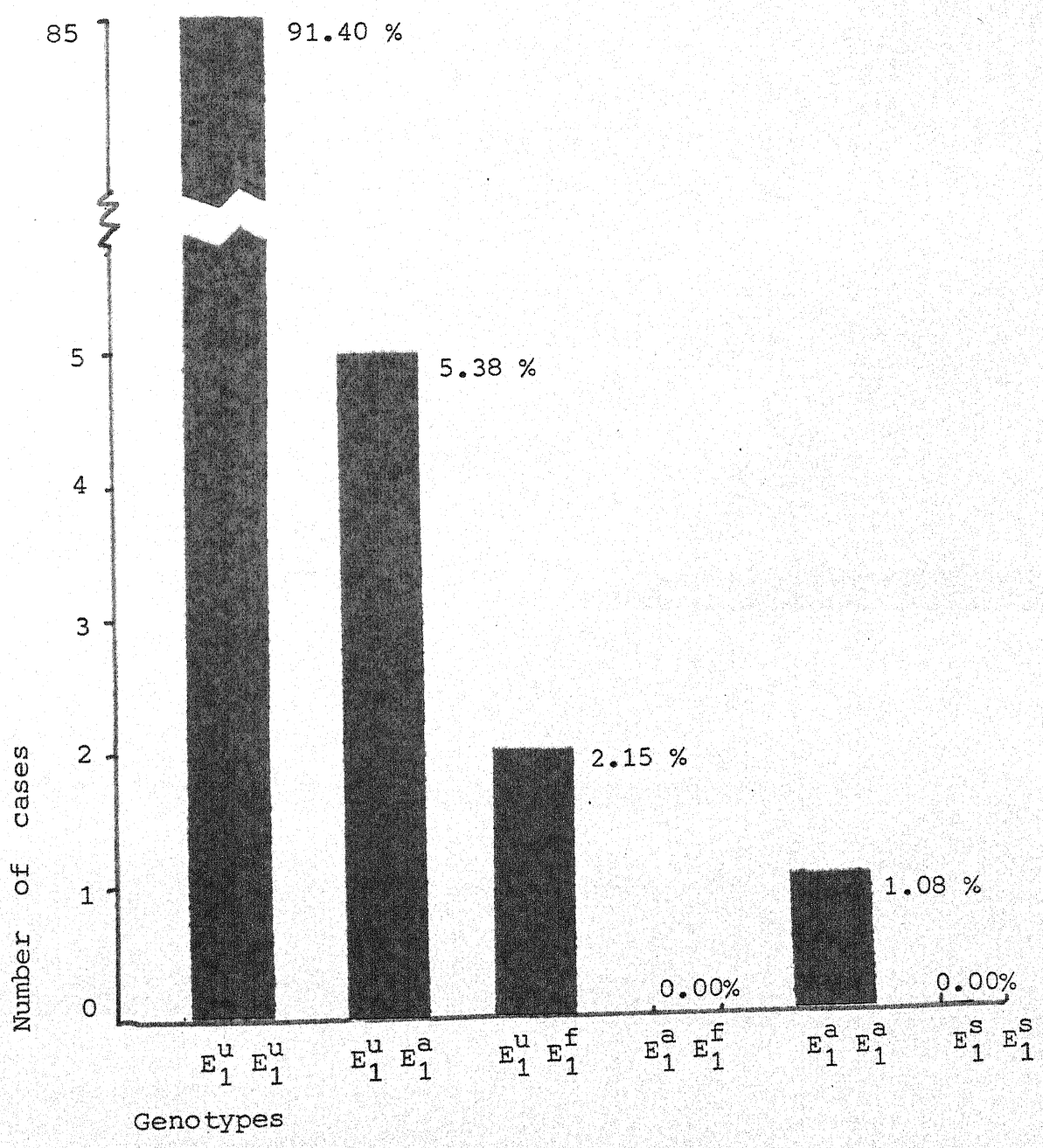


Fig. 13 - DISTRIBUTION OF PERIODS OF APNOEA IN THE STUDY.

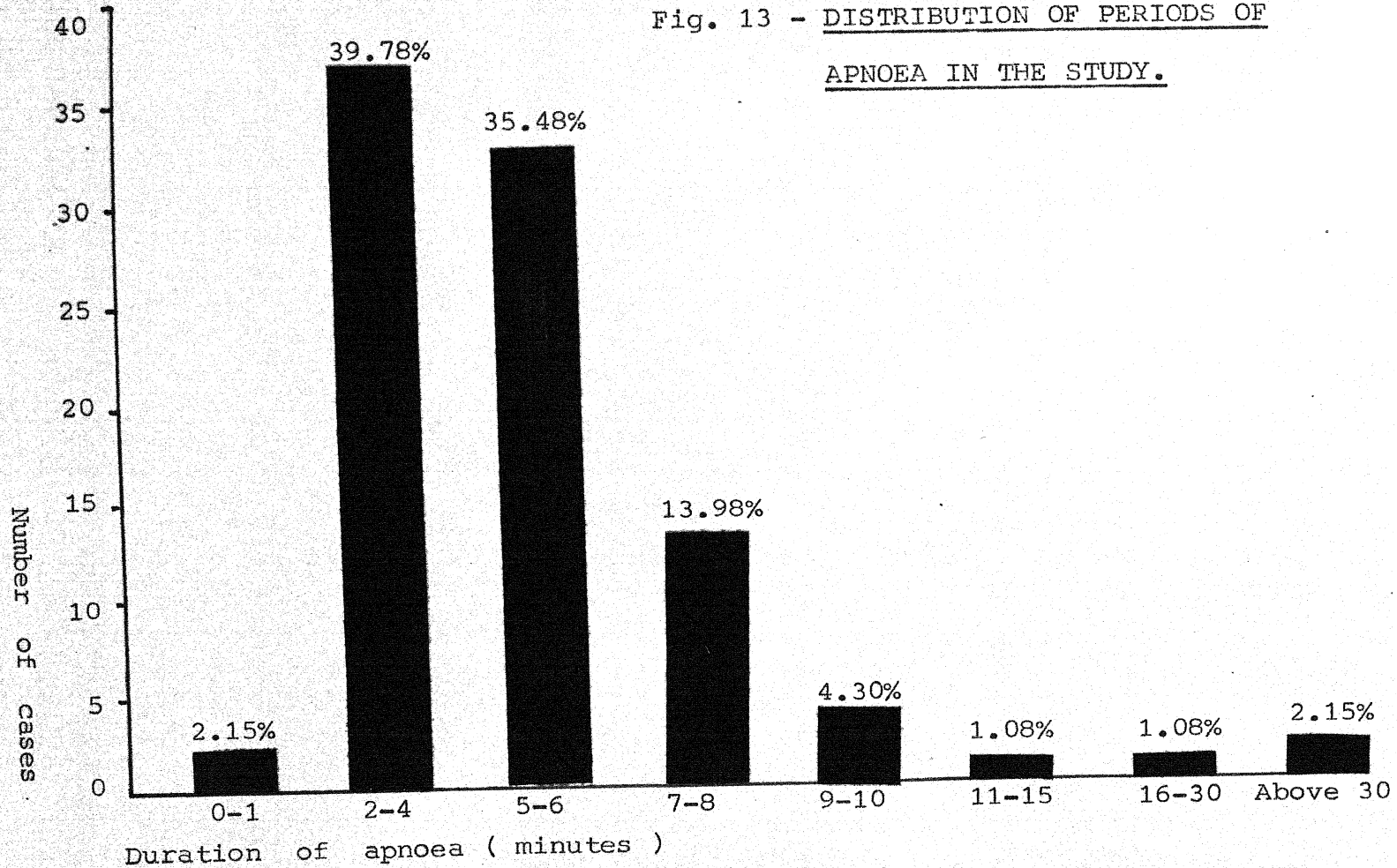


Table 23 - Distribution of periods of apnoea in the study.

Sl. No.	Duration of apnoea (minutes)	Number of cases	Percentage distribution
1.	0 - 1	2	2.15
2.	2 - 4	37	39.78
3.	5 - 6	33	35.48
4.	7 - 8	13	13.98
5.	9 - 10	4	4.30
6.	11 - 15	1	1.08
7.	16 - 30	1	1.08
8.	Above 30	2	2.15
	Total	93	100.00

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DISCUSSION

D I S C U S S I O N

Suxamethonium sensitivity is not a disaster but it can be quite inconvenient. If patients' cholinesterase status is known preoperatively, adequate precautions can be taken before hand.

It has been pointed out that atypical cholinesterase is the main cause of prolonged apnoea (editorial, Lancet, 1973). For the genetically normal cholinesterase Evans et al. (1952), Argent et al. (1955) and Vickers (1963) have shown that a substantial quantitative reduction of enzyme must occur before any significant prolongation of the apnoea takes place. Bourne et al. (1952), in their study of 546 cases noted the presence of lower serum cholinesterase levels in persons having delayed recovery after suxamethonium :

	Mean ± Standard Error (units of Callaway et al.)
Delayed recovery -	38.3 ± 6.8 units/ml.
Normal recovery -	88.5 ± 6.9 units/ml.

Vickers (1963) stated that a less than 25 % enzyme activity was required to produce a significant prolongation of the apnoea; a level of less than 50 % has been stated as necessary by Whittaker (1980). Lievre (1980) is of the opinion that a low cholinesterase level is not an infallible indicator of complications due to prolonged apnoea.

In contrast, Kalow and Gunn (1957) had found the logarithmic relationship between dose and response to be linear

with normal distribution of the logarithms of the dose required to produce a standard effect and the logarithms of the duration of apnoea after standard doses. In agreement to this King and McQueen, in a guest discussion in an article by McLaren and Moffit (1976), state that as a generalisation, the lower the cholinesterase activity and the dibucaine number and fluoride number of a patient, the more prolonged is the response to succinylcholine. They have also explained the reason behind the failure of consistency of this relationship as being technically incorrect estimations.

Keeping these factors in mind, one of the aims of the present study was to try an establish, if possible, a relationship between serum cholinesterase levels and the duration of post-suxamethonium apnoea in the local population. On calculating the correlation coefficient between these two factors in patients of the normal genotype, it has been found that, $r_1 = -.4766392$, obtained for them indicated that a limited degree of negative correlation exist between cholinesterase and the duration of apnoea in this study. Thus, as a generalisation, when cholinesterase value droped, the duration of apnoea increased and vice-versa (Fig. 8).

A similar correlation was studied between dibucaine numbers and fluoride numbers with the duration of apnoea. For dibucaine number vs. duration, $r_2 = -.7152$ was obtained. This suggested a stronger negative correlation with duration than that of cholinesterase activity (Fig. 10).

Fluoride number was also found to be negatively correlated to duration of action of suxamethonium (Fig. 11), implying an increase of apnoea in response to a fall in fluoride number (r_3 = -.6358).

Thus although a generalised relationship between quantitative reductions of cholinesterase activity and the duration of action of suxamethonium in normal individuals has been demonstrated, it is of importance to note that of the four cases in the study who had an apnoea of over 10 minutes (Fig. 13), 3 (75 %) were of abnormal genotypes (two $E_1^u E_1^a$ and one $E_1^a E_1^a$). The single normal homozygote ($E_1^u E_1^u$) having an apnoea over 10 minutes had an enzyme level of 30.0 units by the Steinitz et al. method - thus showing a 61.46 % reduction of its level as compared with the control group.

Thus although prolongation of apnoea is found to be generally associated with low cholinesterase levels, of the 4 cases having pronounced prolongation 3 had abnormal genotypes and the 4th a normal genotype had greater than 50 % quantitative reduction of enzyme. This is in accordance with Evans et al (1952), Argent et al. (1955), Vickers (1963) and Whittaker (1980).

The second aim of this study was to investigate the possible presence of any regional bias regarding cholinesterase levels and the incidence of atypical, fluoride resistant and silent genes, with a view of establishing an existing relationship

if any, with relegious status.

Studies have indicated a probable absence of E_1^a gene in Japanese, Eskimos, South American Indians and a rare presence in Negroes, Austratian aboriginees, Filipinos and oriental populations other than Japanese (Lubin et al., 1971), and the absence of E_1^a gene in Zimbabwe - Rhodesia Africans (Whittaker et al. 1976).

A high incidence of the silent allele in the Alaskan Eskimos and some Caucasian South Africans was noticed and to this list were later added the Vsyas of Andhra Pradesh (Rao, 1979). The discovery of the presence of such ethnic groups in other places prompted an investigation into the genotype characteristics of Bundelkhand Region.

The incidence of E_1^s gene observed in the study was zero. This however may be a fallacious conclusion since though it is to recognise the E_1^s homozygote due to little or no enzymic activity, the detection of the E_1^s heterozygote through simple laboratory estimations is not so simple (Whittaker, 1980). Thus it is quite possible that some heterozygotes for the usual and silent genes may have been missed in this study.

The maximum number of cases in the present project were usual homozygotes (E_1^u E_1^u). These were 91.40 % in number. The heterozygotes E_1^u E_1^a were 5.38 % and $E^u{}_1E_1^f$ were 2.15 %. There was one (1.08 %) atypical homozygote in the study (Fig. 12). An attempt to compare these incidences with these reported by

workers such as Viby - Mogensen et al, (1978) is not correct since the latter report was a patient showing prolonged suxamethonium apnoea while the patients induced here are from a study population which was not limited to ones showing suxamethonium sensitivity only.

Out of the 8 genetically abnormal patients, 7 (87.5%) were Hindus and 1 (12.5%) was a Muslim. The preponderance of Hindus among patients of abnormal genotypes can be explained by the 10.63 : 1 Hindu preponderance present in the whole study population. These 8 cases were not related to each other and belonged to different areas of Bundelkhand.

Individual Cases presenting with prolonged apnoea-

Case No. 3 :

This was a sturdy 60 kg. male aged 35 years. He underwent surgical toilet and debridement of a wound caused by an accidental injury. On examination he was found to be a healthy person. There was no personal or family history of any operation or illness.

After a 75 mg. intravenous dose of suxamethonium he was intubated. Apnoea persisted for a period of 35 minutes during which time he was well ventilated with oxygen and nitrous oxide. Thereafter, small flickering movements appeared in the reservoir bag.

A preoperative blood sample yielded the following data after estimation. Serum cholinesterase activity of 50.5 Steinitz units/ml.; D.N. = 49; F.N. = 44. Suggested genotype : E_1^u E_1^a.

On further enquiries and verification it was learnt that he had received and i.m. injection of 100 mg. Pethidine alongwith 25 mg. promethazine 2 hours ago in the emergency department. Central respiratory depression as a contributory factor to apnoea is a possibility with this case.

Case No. 24 :

A well built 61 kg. Muslim male aged 35 years who underwent a gastrojejunostomy. His blood sugar, urea, Hb., T.L.C., D.L.C. and urine investigations were all within normal limits.

He displayed a 40 minutes suxamethonium apnoea after 75 mg. i.v. suxamethonium.

Serum cholinesterase value of 27.2 units/ml. were seen. D.N. 18 and F.N. 30 establish that this case was an atypical homozygote.

An earlier exploratory laparotomy under G.A. 7 years ago was uneventful. It could not ve determined whether suxamethonium was used or not. There was no family history of any operations.

Case No. 66 :

A 22 years Hindu female who underwent resuturing of a burst abdomen 13 days after a Caesarean section. She was 56 kg. in weight, had a moderate degree of anaemia and was of poor general condition. Her serum cholinesterase level (30.0 units/ml.) was the lowest seen in any of the normal homozygotes studies which she was (D.N. 75; F.N. 68).

After a 60 mg. i.v. dose of suxamethonium, apnoea lasted 15 minutes. No other factor known to cause prolongation of apnoea such as hyperventilation was present. During her Caesarean section 13 days before she was administered suxamethonium (75 mg. i.v.). She had an apnoea of 11.5 minutes. No cholinesterase estimations were done then.

It is suggested that prolonged apnoea in this case was a result of a nearly 62 % reduction in enzyme levels due to the preexisting anaemia, shock and the post-partum period.

Case No. 93 :

A 25 years old 46 kg. Hindu lady in poor general condition and with severe anaemia. She received 50 mg. i.v. suxamethonium during a hysterotomy and ligation. Apnoea lasted for 25 minutes. Her serum cholinesterase activity was 36.2 units/ml.; D.N. was 59 and F.N. 42.

One of the greatest difficulty in any study of the enzyme serum cholinesterase is the virtual inability to compare the results those obtained by with any other method due to different reaction conditions (Bowers and McComb, 1970; Wetstone and La Motta, 1965; Michel, 1961). It is therefore necessary to compare results on a relative basis (Michel, 1961).

It was demonstrated by Milhorat, 1938 that low enzyme levels were present in cases of malnutrition and anaemia. These findings were supported by the work of Faber,(1943) and Vorhaus and Kark (1953) for malnutrition cases and by Scudamore et al.

(1951) and Sawitsky (1949) for chronic anaemias. In the present study too, low levels of enzyme activity have been obtained for the anaemia and malnutrition cases. Barclay (1973) obtained a high incidence (83 %) of low serum cholinesterase levels in study of 302 cases of malnutrition. In the present study, the mean enzyme level for the anaemia and malnutrition cases is the lowest for any of the disease groups studies.

It is worth noting here that the presence of sub-clinical liver dysfunction or occult biliary disease is widely acknowledged (Isselbacher, 1980). A variety of conditions such as anaemias long standing malnutrition, congestive cardiac failure and infective hepatitis may result in a symptomatic liver dysfunction and may thus reflect on the serum cholinesterase levels in spite of the absence of any overt hepatic or biliary disorder.

A mean cholinesterase level of 57.77 units/ml. was obtained in cases of pregnancy in the present study. This reduction of the enzyme level from the normal healthy non-pregnant adult was of the order of 25.78 %. This is closely comparable to the values of the reductions of enzyme activity observed during pregnancy which are 18 % (Robertson, 1966); 21 % (Hazel, 1955); 25 % (Levine and Hoyt, 1949); 28 % (Schnider, 1965) and 30 % (Redderson, 1973). It is in contrast to the findings of Hall and Lucas (1937) and Meade and Rosalski (1963) both of whom found no change in enzyme activity during

pregnancy and early puerperium.

In early post-partum cases a reduction of 32 % in the normal enzyme levels on the third day of puerperium was noted by Hazel and Monier (1971). A figure of 33 % reduction was observed by Whittaker (1980) in Blitt et als' (1977) study on the third post.partum day.

All the cases in the post-partum group underwent serum cholinesterase estimations on the third day of puerperium. A value showing 34.80 % reduction from the non-pregnant adult activity was noticed. This compared closely with the trends observed by Hazel and Monier (1971) and Blitt et al. (1977).

A higher incidence of prolonged apnoea in heterozygotes was seen for the pregnancy group (50 %) as compared with the non-pregnant healthy adult (0 %) this can be explained by Whittakers' (1980) opinion that a higher incidence of prolonged apnoea in heterozygotes is seen in pregnant individuals as compared to healthy non-pregnant adults. This is due to the low enzyme characteristics of heterozygotes.

When seen in the control group, where no other factor was present to affect the serum cholinesterase levels none of the 2 heterozygotes (one of E_1^u E_1^a type) showed an apnoea above 10 minutes. Kalow and Gunn (1957) had also observed that a heterozygote for a typical serum cholinesterase does not have greatly prolonged responses to succinylcholine.

In the dehydration group was encountered the longest mean duration of apnoea (9.22 minutes). Contributing towards this was a 35 minutes apnoea in a case heterozygous for the usual and atypical cholinesterase. Never the less, mean cholinesterase level of this group a patients was lower by a highly significant degree from the control group.

Mean cholinesterase levels of the pregnancy, post-partum, malignancy, liver and biliary disease, anaemia and malnutrition groups were all reduced by highly significant degrees from the mean level of control cases. This is in agreement with findings of most workers.

Reduced enzyme levels for liver diseases were observed by Jones and Stadie (1939); McArdle (1940); Foldes (1940); Lehmann et al. (1962) and Hunter (1966). Lehmann et al. (1962) found a level of 8 - 59 units/ml. (as compared to a normal level of 60 - 120 units/ml.). Hunter (1966) found a mean enzyme activity of 86 units in the cases with liver and biliary diseases while Foldes (1940) found a mean level of 59 $\pm$ 9.5 units/ml. and Jones and Stadie (1939) found a mean level of 41 units/ml. in advanced tuberculosis and carcinoma. They attributed it to temporary injury to liver. This study shows a drop of enzyme activity in the liver disease group, but not to the extent found by Lehmann et al. (1962).

This was probably due to absence of cases with true parenchymal damage to the liver. The cases of this group in the present study were of chronic cholecystitis predominantly.

Malignancy cases presented a range of enzyme activity from 40.6 - 69.9 units/ml.. There was a lowered activity in malignancy cases studied by Jones and Stadie (1939) and McArdle (1940). No evidence of hepatic metastases was seen in any of the cases in this study. Hepatic metastases had been associated with a still greater drop of enzyme activity as observed by Kaniaris et al. (1979); Ghooi et al. (1980). Wetstone et al. (1960) had suggested the reason for the drop in enzyme activity to be due to the carcinomatous tissue itself which perhaps was thought to be responsible for production of a serum cholinesterase inhibitor (Kaniaris et al., 1979).

As regards the changes in mean cholinesterase activity with the age it was noticed in present study that slightly higher levels are present in children. A fall in activity of the enzyme occurred around puberty and was maintained till 50 years of age after which a subsequent rise to still higher levels was seen in the age groups (51 - 60) years and (61 - 70) years. The significance of this increase in later years was doubtfull since the two groups comprised of 2 and 1 individuals only. Results of present study are comparable to the work of McCance et al. (1949) who noticed a dramatic rise of the cholinesterase levels in the early childhood. Dabew (1970) found a 30 % increase in the activity of enzyme in 3 to 6 year old children followed by a gradual fall to the adult level by puberty. In the oldest two groups the rise of enzyme activity is in contrast to the

observations of Kalow and Gunn (1959) and in agreement to those of Propert and Brackenridge (1976). The values for children in this study (mean age of 11.12years) was found to be 3.64 % higher than the controls.

In cases of obesity, high serum enzyme level was noted by Bery et al. (1954) in study of 354 cases. Thompson and Trounce (1956) also held the obesity present in patients of diabetes to be the cause of reduction of cholinesterase activity in such cases. The present study shows the highest mean cholinesterase level present for any of the groups studied (106.95 units/ml.) have observed. It has also been noticed here that cases of thyroid disorder too had an increased mean cholinesterase level but difference from the controls was not significant. However, one patient (case No. 53) had higher enzyme levels and displayed signs of mild thyrotoxicosis. Although in some of the mental disorders like anxiety and depressive states and schizophrenia (Tod and Jones, 1937 ; Antebi and King, 1962 and Propert, 1979) higher levels of the esterase were found. The higher activity seen in the present study is not significantly different from the control group.

No influence of residential status or diet was seen on the enzyme levels in this study. The alcohol users had slightly higher levels. Alcoholism has been shown to be associated with higher levels of cholinesterase (Vaccarezza and Peltz, 1960).

A non-significant increase of cholinesterase in the adult males as compared with the non-pregnant adult females was seen to be present (Kalow and Gunn, 1959; Wetstone and La Motta, 1965 and Propert and Brackenridge, 1976). These reports are in conformity with the present study. However, the findings are incontrast to Hall and Lucas (1937);Callaway et al. (1951) and Vorhaus and Kark (1953). The present study shows a clear trimodal histribution of the population when the cases were grouped according to dibucaine number. Three distinct genotypes were seen in separate groups (Fig. 14). Our findings regarding the trimodal distribution of the population are in agreement with Whittaker (1980).

An increased mean enzyme level was observed in the routine cases as compared to the emergency cases. However, it was insignificant. In various dietary groups studied, no significant variations of serum cholinesterase were noticed. This is in agreement with the findings of Kaufman (1954).

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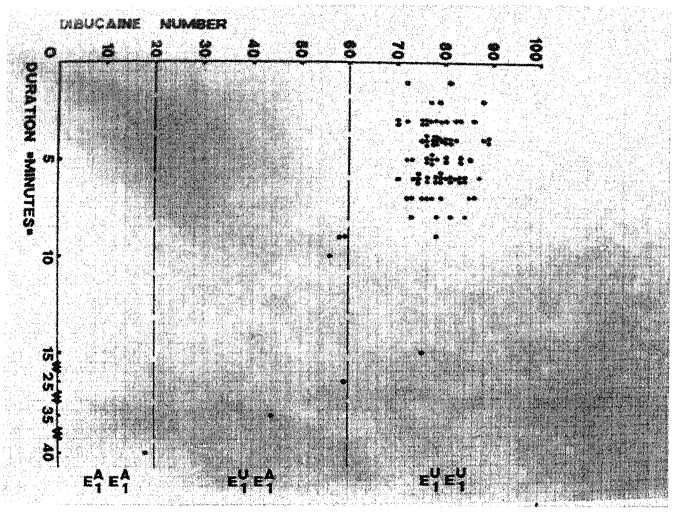

Fig. 14 - SHOWING CLEAR TRIMODAL DISTRIBUTION INTO THREE DISTINCT GENOTYPES WHEN CASES WERE GROUPED ACCORDING TO DIBUCAINE NUMBER.

SUMMARY & CONCLUSION

S U M M A R Y A N D C O N C L U S I O N

The present project was undertaken at the M.L.B. Medical College, Jhansi, from August, 1981 to February, 1982, with the objective to study the quantitative variations of serum cholinesterase in Bundelkhand Region and the relationship to suxamethonium apnoea. The incidence of various genotypes and the association of abnormalities with regional or religious status were also investigated.

For this purpose, 93 patients belonging to both sexes and various age groups from (0-10) years to (61-70) years were divided into - Control group (26), Anaemia and malnutrition (21), Pregnancy (15), Post-partum (6), Dehydration (9), Malignancy(4), Liver and biliary diseases (3), Mental disorders (3), Thyroid disorders (4), Obesity (2) and Children (6). Due to the presence of multiple factors in some patients they were placed separately under more than one group.

Control cases were also divided according to age, sex, alcohol intake, diet, residential , socioeconomic and smoking status so as to study the influence of these on the enzyme levels.

An elaborate history and details of physical examination were recorded for each case. Blood sample were drawn in each case and serum was separated and stored.

The cases were induced with pentothol followed by

required dose of suxamethonium and apnoea was recorded.

The serum cholinesterase activity, Dibucaine number and fluoride number were estimated by the Steinitz et al. method.

On the basis of the observations made during the present study, the findings can be summarised as follows :-

1. The mean serum cholinesterase activity of normal healthy adults (Controls) was 77.84 ± 8.10.

2. Statistically, highly significant reductions of the mean enzyme level from the control group was seen in the following conditions or diseases - anaemia and malnutrition, pregnancy, post-partum, dehydration, malignancy and liver and biliary diseases.

3. Statistically significant elevations of the mean enzyme activity above the controls were observed in cases of obesity and thyroid disorders.

4. Variations of the enzyme activity in group of children and mental disorder were not significant .

5. Highest cholinesterase values among the various groups were observed with cases of obesity, while the lowest were recorded with anaemia and malnutrition.

6. The duration of apnoea following a 1 mg./kg. of body weight of suxamethonium ranged from 1.5 minutes in cases of obesity to 9.22 minutes in cases of anaemia and malnutrition.

7. A limited degree of negative correlation was present between the serum cholinesterase activity and the duration of apnoea.

A similar relationship was found when the duration of apnoea was correlated with either Dibucaine number or Fluoride number.

8. Incidence of prolonged apnoea was 4.30% (4 cases).

Out of these, 3 cases (75 %) were of genetic abnormality (two $E_1^u E_1^a$ and one $E_1^a E_1^a$), while 1 (25 %) was of quantitatively decreased cholinesterase level.

9. The single case of prolonged apnoea with a quantitative decrease of the cholinesterase level was seen to have a 61.46 % reduction in comparision to normal healthy controls.

10- In this study 91.40 % population was usual homozygote ($E_1^u E_1^u$), 5.38 % was heterozygote for the usual and atypical genes ($E_1^u E_1^a$), while 2.15 % was heterozygote for the usual and fluoride resistant gene ($E_1^u E_1^f$). Only one atypical homozygote ($E_1^a E_1^a$)was present constituting 1.08 % of the study population.

11. Though a preponderance of Hindus was found among the abnormal genotypes the study could not reflect any significance due to a 10.63 : 1 ratio of Hindus to Muslims in the study population.

12- A higher incidence of prolonged apnoea in cases of heterozygotes was seen in the pregnancy group in comparision to normal subjects (Controls). However, apnoea was not necessarily prolonged in atypical heterozygotes without any other coexisting factor as has been observed with the heterozygotes of control group.

13. The study reflected a raised serum cholinesterase activity in children and old age (above 50 years). However, the findings were statistically inconclusive.

14. A distinct trimodal pattern regarding Dibucaine number distribution was noted in the study population.

15. Although single variations in the enzyme activity in persons hailing from various areas of Bundelkhand and other regions were noticed, these observation were statistically insignificant.

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APPENDIX

A P P E N D I X - I

A STUDY OF THE RELATIONSHIP BETWEEN SUXAMETHONIUM APNOEA AND SERUM CHOLINESTERASE IN THE LOCAL POPULATION.

Case No. -

M.R.D. No. -

Name -

Age/Sex -

Relegion -

Father's name -

Address - House No. -
Mohalla -
Village -
Tehsil -
P.O. -
District -

Occupation -
If farmer, fertilizer salesman, anti-malaria worker, gardener - Insecticide or pesticide used -

Socioeconomic status -
Low class/Low middle class/High middle class/ High class.

Ward/Bed No. -

D.O.A. -

Diagnosis (Clinical) -

Diagnosis (Postoperative) -

Diagnosis (Pathological - if done) -

Date of operation -

Surgeon -

Anaesthesiologist -

Complaints	Duration

H/O Present Illness -

Personal History :

Food Habits - High protein e.g. Milk, Meat or Jowar, Bajra eater.
Habits -

	Type/Brand	How much	Since when
Alcohol -			
Smoking -			
Drugs -			
Any other -			

Family History :

* Any operations in the family -
* Deaths during operations in the family -
* Hospital at which such operation and/or death occured -

Past History :

(i) History of drug reaction or allergy

(ii) Worms in stool/Bleeding from any site

(iii) Any Liver disease

(iv) Any Heart disease

Any drgus taken - e.g. Procainamide
Quinidine
Lignocaine
Any other

(v) Malignancy

Any treatment for Malignancy

* Drugs taken -
* Radiation undergone -

(vi) Kidney Disease

(vii) Eye condition - * Glaucoma etc.

* Drugs taken or used (e.g. Phosphorilene eye drops etc.)

(viii) Any previous operation -

* What operation - done -
* Where was it performed -
* Any abnormalities during anaesthesia &/or operation -

Gravida and Parity states :

G _ _ _ P _ _ _ A _ _ _

L.C.B. or last abortion -

If aborted, at what stage of pregnancy -

L.M.P. -

EXAMINATION OF THE PATIENT :

Weight -

Built -

Nutritional status (Cachexia ?, undernourished ?) -

Mental state -

General Condition -

Pulse -

B.P. -

R.R. -

Pallor -

Cyanosis -

Clubbing -

Oedema (including facial oedema) -

Jaundice -

Temperature -

Hydration -

Lymph Nodes -

Dentition - formula -

loose teeth -

pyorrhoea -

Thyroid swelling -

Skin - any skin disease (eczema, psoriasis etc.) -

Cardiovascular System :

Central Nervous System :

Respiratory System :

Abdomen : Liver -
Spleen -
Kidney -
Ascitis -
Miscellaneous -

Spine :

INVESTIGATIONS DONE :

Blood : T.L.C. -
D.L.C. - P -
L -
E -
M -
B -
Hb. -
E.S.R. -
B.T., C.T. -
L.F.T. -
P. ChE. -
Dibucaine No. -
Blood Urea -
Blood Sugar -
Blood Group -

Urine : Complete urine examination -

Others :

ANAESTHESIA :

Anaesthesia given -
Suxamethonium used for (Purpose) -

Any preoperative Blood Transfusion with time -

Date of collection of Blood -

Premedication -

Induction -

Suxamethonium given at (time) -

Suxamethonium dose -

Suxamethonium apnoea started at (time) -

Respiration first returned at (time) -

Duration of apnoea (by stopwatch) -

Any other relaxant used -

* in what dose -
* time of administration of other relaxant -
* Prostigmine given -

Blood Transfusion given or not -

* Amount of Blood given -
* Time of administration of Blood -
* Date of collection of Blood -

Any second dose of Suxamethonium used - Yes/No

Second dose of suxamethonium -

Second dose given at (time) -

Apnoea started at (time) -

Respiration now returned at (time) -

Duration of apnoea (by stopwatch) -

A P P E N D I X - I I

CRITERIA PREPARED FOR THE SOCIOECONOMIC CLASSIFICATION OF THE STUDY POPULATION.

High class (1):

This group comprised of patients coming from a family with a per capita income of more than Rs. 500=00 per month or a total family income of more than Rs. 3000=00 per month.

High-middle class (2):

This includes patients with a per capita income from Rs. 351=00 to Rs. 500=00 per month, or a total family income of Rs. 2001=00 to Rs. 3000=00 per month.

Low-middle class (3):

Cases with a per capita income between Rs. 250=00 and Rs. 350=00 per month or a family income between Rs. 1000=00 and Rs. 2000=00 per month were grouped here.

Low class (4):

Cases with a per capita income of Rs. 249=00 or less per month, or a total family income of Rs. 999=00 or less per month were included here.

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